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TITLE: Wnt Signaling in Prostate Cancer Bone Metastases

PRINCIPAL INVESTIGATOR: Wachiraphan Supsavhad

CONTRACTING ORGANIZATION: The Ohio State University
Columbus, OH 43210

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14. ABSTRACT Ace-1-Dkk-1, a canine prostate cancer overexpressing Dkk-1 was used in this study to investigate how enhanced Wnt/JNK signaling could alter tumor growth, metastasis and the bone microenvironment. Evidence was found that Dkk-1 up-regulated the non-canonical Wnt/JNK pathway resulting with downstream alterations in gene expression important for osteoblast stimulation, cell proliferation and epithelial-to-mesenchymal transformation of cancer cells. Inhibition of non-canonical Wnt/JNK signaling using SP600125 (JNK inhibitor) significantly increased the mRNA expression of genes that induced bone formation as well as decreased osteoclastic bone lysis in vitro. Dkk-1 increased tumor volume in mice. When mice were injected subcutaneously with Ace-1-Dkk1, treatment with SP600125 significantly reduced tumor size and altered tumor cell morphology. However, treatment with SP600125 did not alter tumor size in mice that were injected intra-tibially with Ace-1-Dkk1. Inhibition of non-canonical Wnt/JNK signaling using SP600125 resulted in decreased tumor volume but did not alter tumor size in the bone.					
15. SUBJECT TERMS Prostate cancer, Bone Metastasis, Wnt signaling					
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1.Introduction

Prostate cancer is the second most common cancer in men worldwide [1]. The incidence of this cancer increases with age [1]. The majority of patients with prostate cancer die from metastatic disease, and bone is the most common metastatic site [2]. Osteoblastic metastases are the major phenotype of bone metastases in prostate cancer [3]. It has been proposed that tumor-derived Wnts stimulate bone formation in metastatic cancers through the Wnt signaling pathway [4, 5]. The Wnt signaling pathway is classified into two major categories; 1) canonical and 2) non-canonical signaling pathways. Canonical Wnt signaling is speculated to play a role in osteoblast differentiation and the regulation of osteoblastic bone metastases [6]. Wnt proteins form complexes with the transmembrane protein Frizzled (FZ/Fzd) and low-density lipoprotein (LDL) receptor-related protein 5/6 (LRP5/6) to inhibit the degradation of β -catenin in the cytoplasm. The protected β -catenin translocates to the nucleus and up-regulates genes that are needed for osteoblastogenesis [7]. On the other hand, how non-canonical Wnt signaling plays a role in bone-metastatic prostate cancer is unclear. The Wnt/JNK pathway (Wnt/RAC and RHO) is 1 of 5 sub-categories of the β -catenin-independent non-canonical Wnt pathway [8]. In this pathway, Wnt proteins transduce their signal independently from LRP5/6 to activate c-Jun N-terminal kinase (JNK) protein and its downstream targets [8]. Dkk1 is one of the osteoblastogenesis inhibitors, which not only blocks the canonical-Wnt pathway, but also activate non-canonical Wnt signaling. [9-11]. High levels of Dkk1 were commonly detected in early stage prostate cancers and decline after cancers progress [12]. The association between high serum Dkk-1 and short survival time in prostate cancer patients was reported [13]. However the role of Dkk1 in canonical and non-canonical Wnt signaling pathways in prostate cancer remained unclear. Previous studies in dog prostate cancer demonstrated that alteration of Dkk-1 expression in a dog prostate cancer cell line changed the metastatic phenotype and tumor growth in vivo [6]. In this study, we hypothesized that Dkk1 would inhibit canonical Wnt signaling and activate the non-canonical Wnt/JNK pathway in prostate cancer. Inhibiting non-canonical Wnt/JNK signaling using a JNK inhibitor (SP600125) changed tumor growth and the bone metastatic phenotype of prostate cancer. Ace-1-Dkk-1, a canine prostate cancer overexpressing human Dkk-1, previously developed in our lab was used in this study to investigate the role of Wnt signaling in prostate cancer growth and bone metastases.

2.Keywords

Prostate cancer, bone metastases, Wnt signaling, Dkk1

3.Accomplishments

What were the major goals of the projects?

Aim 1. Determine the role of the canonical Wnt and the non-canonical Wnt/JNK signaling pathway on prostate cancer cell proliferation in vitro.

Aim 1.1: Investigate the expression of human Dkk-1, non-canonical Wnt/JNK and canonical Wnt signaling pathways, and proliferation in the Ace-1 and the human Dkk-1-transfected Ace-1 (Ace-1-Dkk-1) cell lines.

Aim 1.2: Investigate the gene expression effect of inhibiting the non-canonical Wnt/JNK signaling pathway in Ace-1-Vector and Ace-1-Dkk-1 cell lines.

Inhibit non-canonical Wnt/JNK signaling pathway in Ace-1-Vector and Ace-1-Dkk-1 cell lines with the JNK inhibitor (SP600125).

Aim 2 Determine the role of the canonical Wnt and the non-canonical Wnt/JNK signaling pathway on prostate cancer cell proliferation in vivo.

Aim 2.1: Investigate the effect of the canonical Wnt and the non-canonical Wnt/JNK signaling pathway on tumor growth in mice with Ace-1-Vector and mice with Ace-1-Dkk1 tumors, respectively.

Aim 2.2: Determine the effect of a JNK inhibitor (SP600125) on tumor growth and the expression of JNK-related genes in mice with Ace-1-Vector and in mice with Ace-1-Dkk1 tumors.

Aim 3. Measure the effect of the canonical Wnt and the non-canonical Wnt/JNK pathways on bone microenvironment in prostate cancer.

Aim 3.1: Investigate the effect of the canonical Wnt and the non-canonical Wnt/JNK signaling on bone resorption ex vivo.

Aim 3.2: Determine the effect of inhibiting the non-canonical Wnt/JNK signaling pathway on tumor growth and bone resorption in vivo.

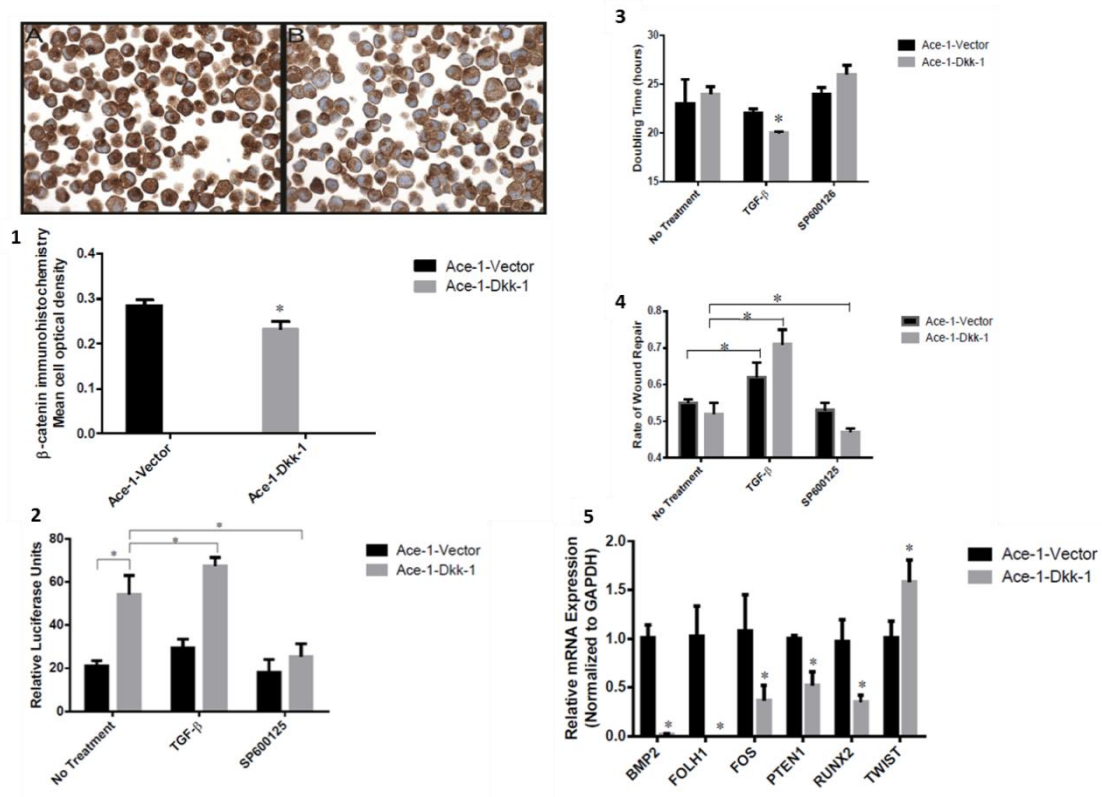
Timeline

Plans	2015									2016						
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Aim 1.1																
Aim 1.2																
Aim 2.1																
Aim 2.2																
Aim 3.1																
Aim 3.2																
Analyze data																

What was accomplished under these goals?

Aim 1.1: Investigate the expression of Dkk-1, non-canonical Wnt/JNK and canonical Wnt signaling pathways, and proliferation in the Ace-1 and the Dkk-1-transfected Ace-1 (Ace-1-Dkk-1) cell lines in vitro and in vivo.

Dr. Jessica Simmons found that Ace-1-Dkk-1 significantly decreased beta-catenin immunostaining intensity, and AP-1 activity in Ace-1-Dkk1 cells was significantly greater compared to Ace-1-vector cells (Figure 1). AP-1 activity as well as cell migration rate in Ace-1-Dkk-1 cells were significantly decreased after treatment with SP600125 (Figures 2 and 4). Moreover, mRNA expression of bone-related genes, including BMP2 and Rux2, as well as prostate specific membrane antigen gene (FOLH) were significantly decreased in Ace-1-Dkk-1 compared to Ace-1-vector cells (Figure 5).

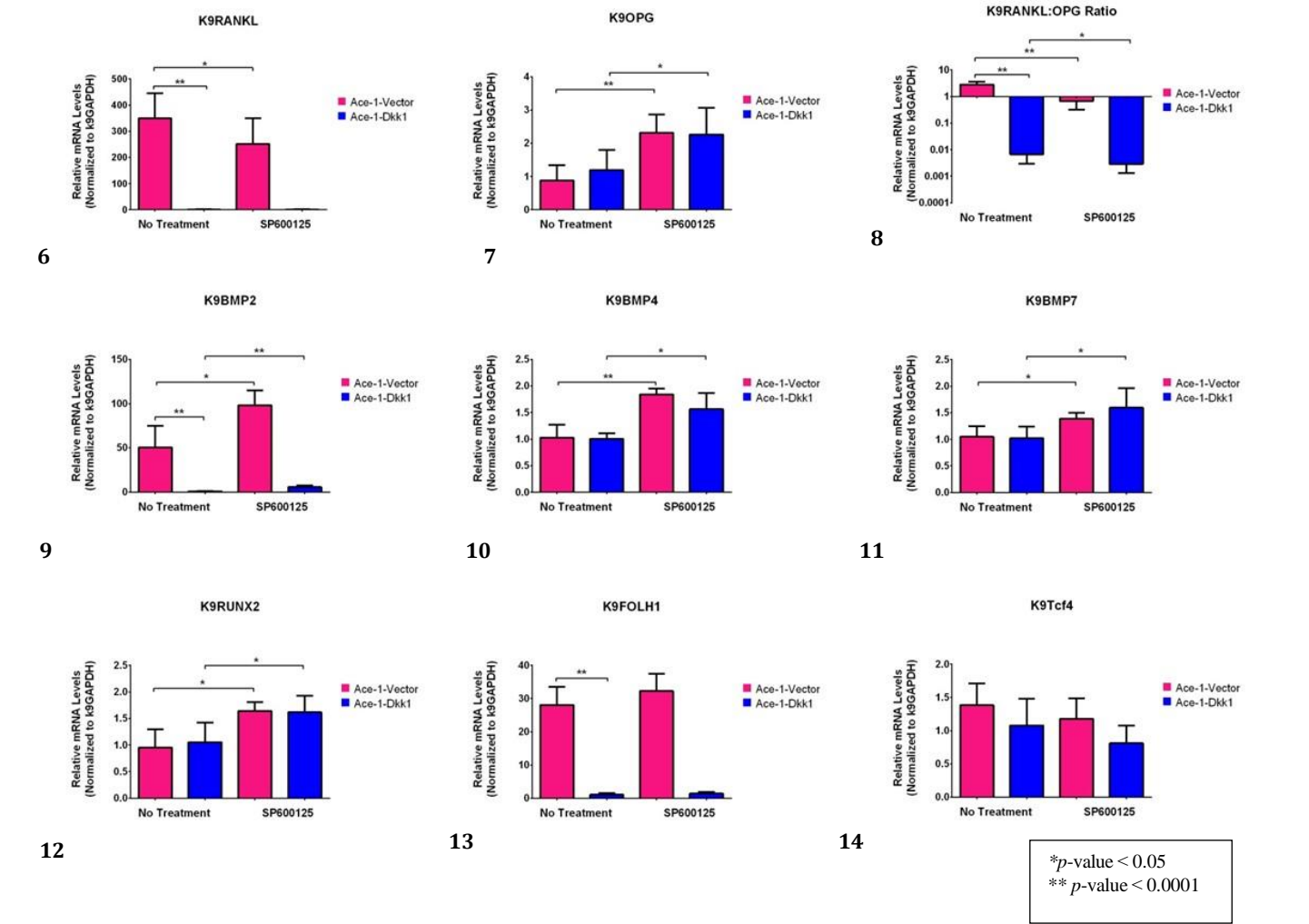


Based on these results, we hypothesized that:

- 1) Overexpression of Dkk1 would cause a switch from canonical Wnt to non-canonical Wnt signaling in Ace-1 cells.
- 2) Inhibiting non-canonical Wnt signaling using a JNK inhibitor or anti-Dkk1-antibody would resume canonical signaling in Ace-1-Dkk1 cells.
- 3) Targeting the Wnt/JNK signaling pathway will provide a novel therapeutic pathway to reduce prostate cancer proliferation and metastasis.
- 4) Treatment of Ace-1-Dkk1 with a JNK inhibitor will decrease tumor growth and metastasis of cancer cells to bone.

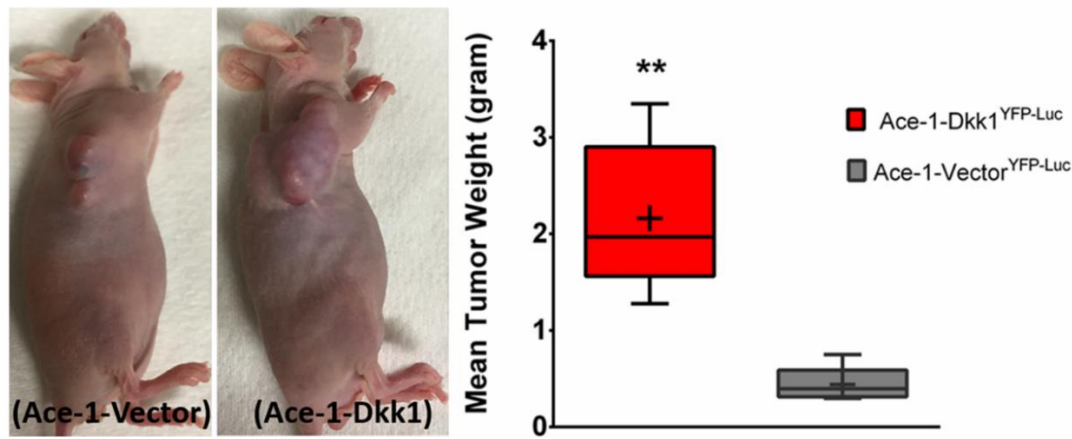
Aim 1.2: Investigate the effect of inhibiting the non-canonical Wnt/JNK signaling pathway in Ace-1-Vector and Ace-1-Dkk-1 cell lines on gene expression.

SP600125 significantly increased the relative mRNA expression levels of genes that related to osteoblast differentiation (BMP2, BMP4, BMP7, and RUNX2) in both Ace-1Dkk-1 and Ace-1-Vector cells (figure 9-12). This drug decreased Tcf4 mRNA in both Ace-1-Dkk-1 and Ace-1-pcDNA cells but had no effect on K9FOLH1 mRNA expression levels (Figure 13, 14). Interestingly, the RANKL: OPG mRNA expression ratio, an indicator of osteolytic bone resorption, was significantly decreased after treatment with SP600125 in both cell lines (Figure 6-8).



Aim 2.1: Investigate the effect of non-canonical Wnt/JNK signaling pathway on tumor growth in mice with Ace-1-Vector and mice with Ace-1-Dkk1 tumors.

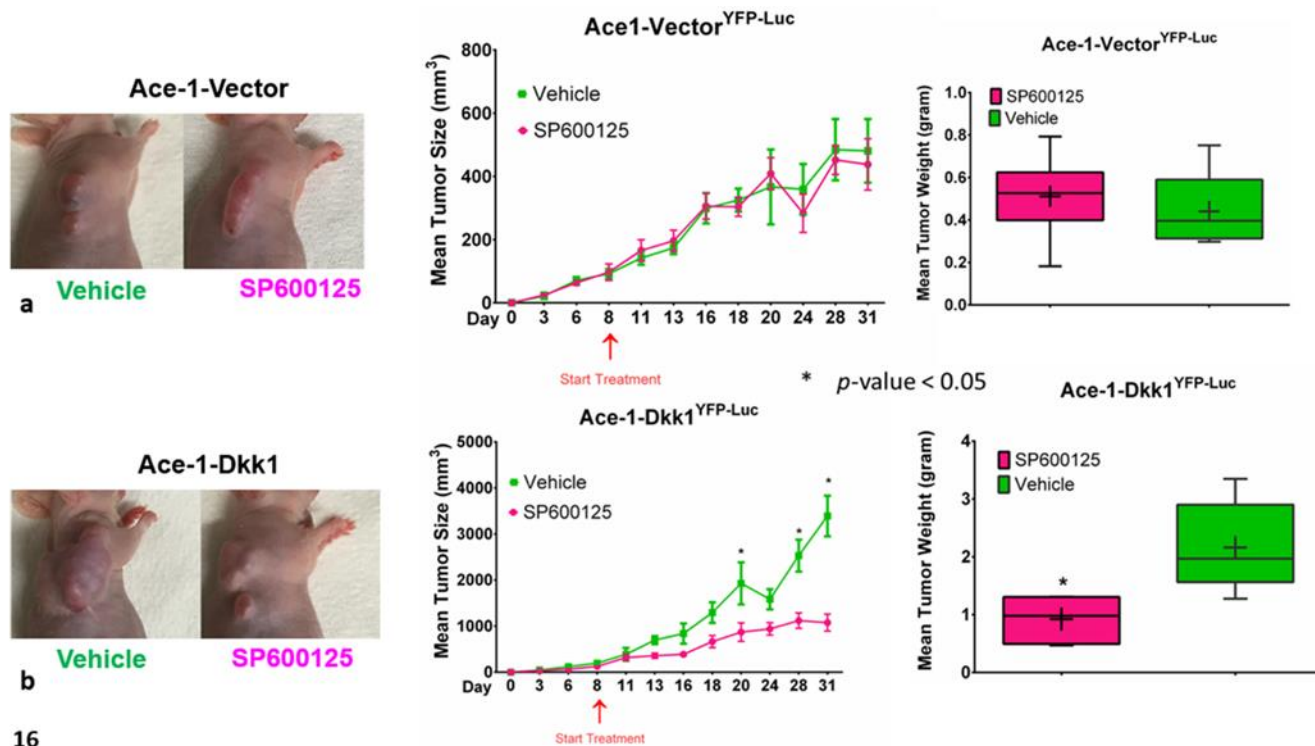
Nude mice with Ace-1-Vector tumors had significantly larger subcutaneous tumors than mice with Ace-1-Dkk1 (Figure 15)

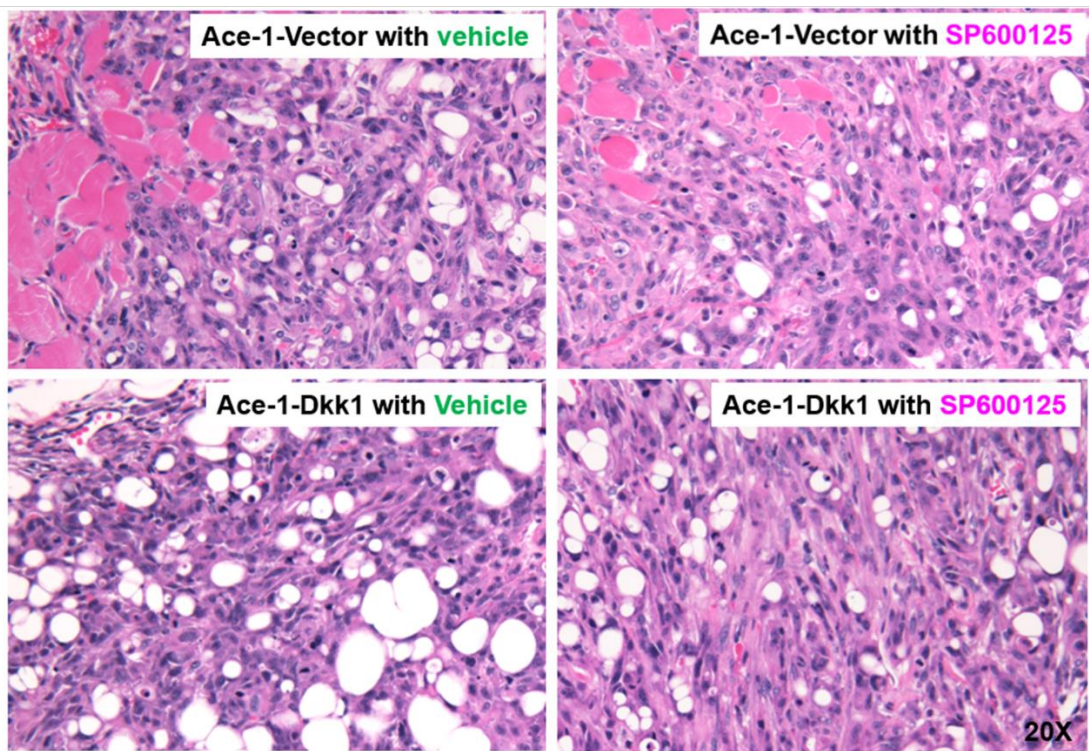


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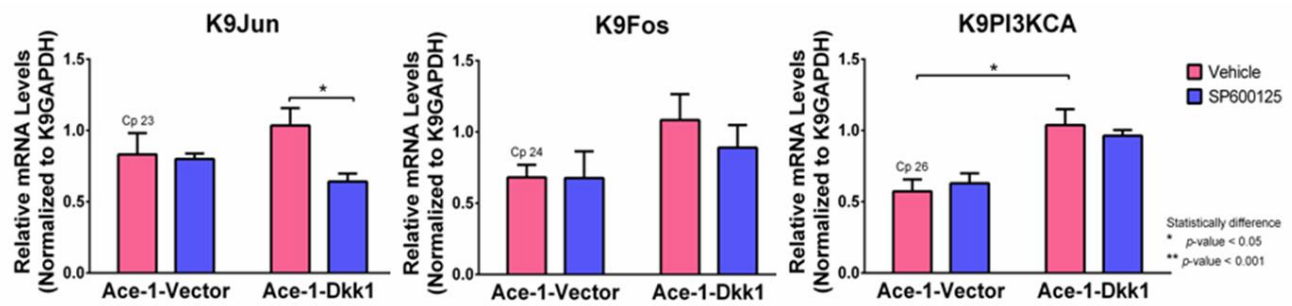
Aim 2.2: Determine the effect of a JNK inhibitor (SP600125) on tumor growth and JNK-related gene expression in nude mice with Ace-1-Vector and nude mice with Ace-1-Dkk1.

Treatment with SP600125 did not alter tumor growth in nude mice with Ace-1-Vector (Figure 16a), but significantly decreased tumor growth in nude mice with Ace-1-Dkk1, starting from 20 days after treatment (Figure 16b). The histomorphology of these tumors demonstrated increased epithelial-to-mesenchymal transition (EMT) after treatment with SP600125 in both Ace-1vector and Ace-1-Dkk1 tumors (Figure 17). The relative mRNA expression levels of the non-canonical Wnt/JNK signaling pathway downstream products (JUN and FOS) tended to increase in Ace-1-Dkk1 tumors (Figures 18 and 19). Treatment with SP600125 significantly decreased JUN mRNA levels in Ace-1-Dkk1 tumors (Figure 18). Ace-1-Dkk1 tumors had significantly greater mRNA expression levels of PI3KCA, an oncogene in several cancers, compared to Ace-1-Vector tumors (Figure 20).





17



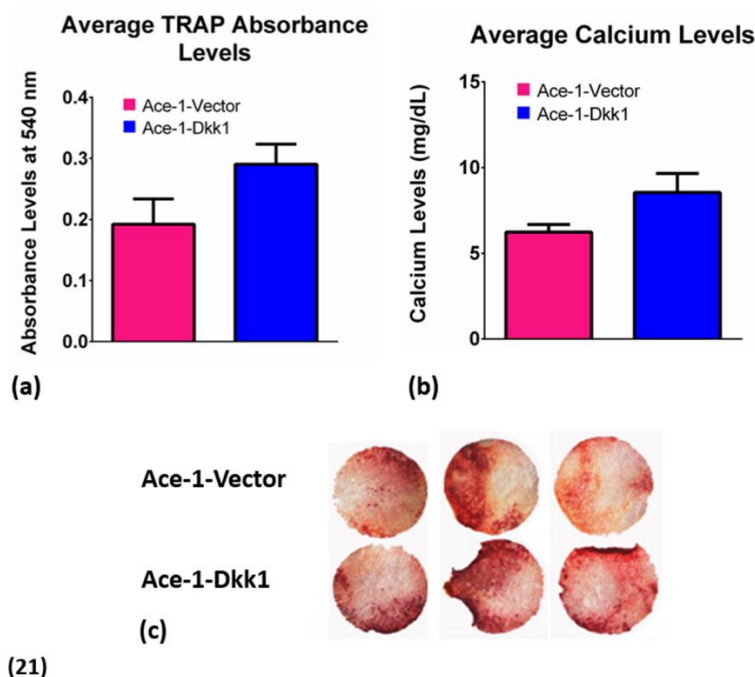
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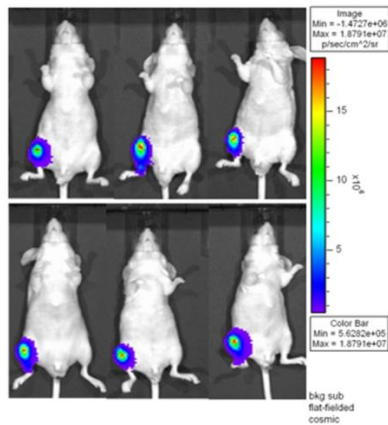
Aim 3.1: Investigate the effect of canonical Wnt and non-canonical Wnt/JNK signaling on bone resorption ex vivo.

Mouse pup calvaria that were co-cultured with conditioned medium of Ace-1-Vector cells tended to have less bone lysis and lower numbers of active osteoclasts compared to the calvaria that were co-cultured with conditioned medium of Ace-1-Dkk1 cells (Figures 21a and c). Co-conditioned medium of Ace-1-Vector medium and calvaria also tended to have lower calcium levels than the co-conditioned medium of Ace-1-Dkk1 medium and calvaria (Figure 21b).

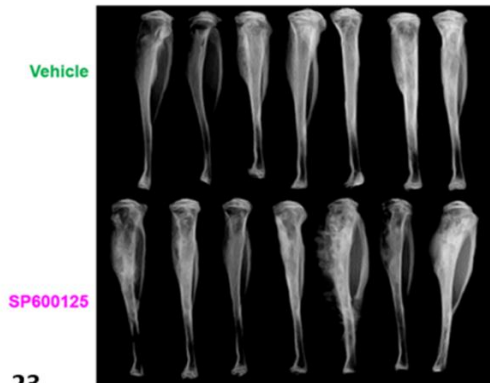
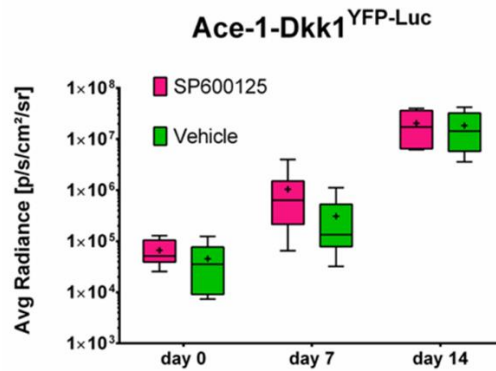


Aim 3.2: Determine the effect of inhibiting the non-canonical Wnt/JNK signaling pathway on tumor growth and bone resorption in vivo.

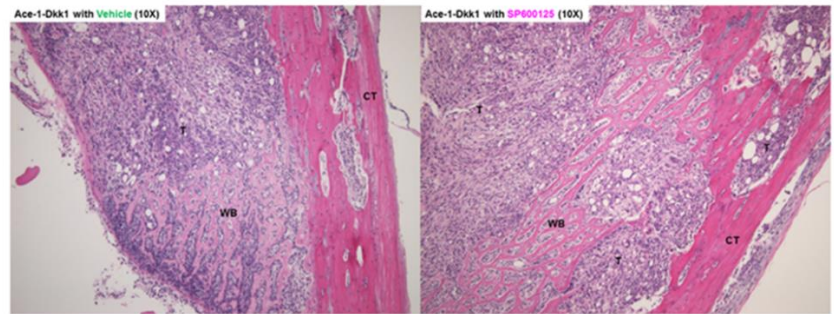
SP600125 did not change tumor growth (bioluminescent signal) in bones of nude mice that were injected with Ace-1Dkk1 cells in their tibias (Figure 22). Increased bone formation on radiographs were observed in Ace-1-Dkk1 bearing-mice with SP600125 treatment compared to Ace-1-Dkk1 bearing-mice in the control group (Figure 23). Histopathological features of Ace-1-Dkk1 tumors in both SP600125 treatment and control groups revealed a similar phenotype of well-differentiated prostate cancer cells with both osteoclastic bone lysis and new bone formation (Figure 24).



22



23



T: tumors, WB: woven bone (new bone) and CT: cortical bone

24

Summary:

Overexpression of human Dkk1 in Ace-1 cells did not only inhibit the canonical Wnt pathway, but also activated the non-canonical Wnt/JNK signaling pathway. Ace-1-Dkk1 subcutaneous tumors were significantly larger than Ace-1-vector tumors. Ace-1-Dkk1 conditioned medium tended to induce more ex vivo bone lysis compared to Ace-1-Vector conditioned medium. SP600125, which inhibited non-canonical Wnt/JNK signaling, did not cause the canonical Wnt signaling to resume in Ace-1-Dkk1 cells. Inhibiting non-canonical Wnt/JNK signaling using SP600125 significantly decreased tumor sizes as well as volumes in Ace-1-Dkk1 but not in Ace-1-Vector tumors. However, SP600125 did not change tumor growth in bone or phenotype of bone-related tumors in Ace-1-Dkk1.

Conclusion:

Human Dkk1 inhibited the canonical Wnt pathway as well as activate the non-canonical Wnt JNK signaling pathway in Ace-1 prostate cancer cells. Ace-1-Vector and Ace-1-Dkk1 cells were useful models for studying the biological and molecular characteristics of canonical Wnt and non-canonical Wnt/JNK signaling pathways in prostate cancer, respectively. High Dkk1 expression might be an indicator for aggressive prostate cancers. SP600125 could be an alternative adjuvant therapy for decreasing tumor size in prostate cancer patients with high levels of Dkk1.

What opportunities for training and professional development has the project provided?

This project has provided me the opportunities to development new laboratory skills related to investigations on bone, including in vitro bone resorption experiments and in vivo bone metastasis studies. In addition, I have been able to spend more time to discuss my work, results and progress with my mentor, Dr. Thomas Rosol.

How were results disseminated to communities of interest?

The research results have been shared at
2014 Annual Meeting of the Society for Toxicologic Pathologists in Washington DC
2016 College of Veterinary Medicine, The Ohio State University graduate student seminar in Columbus OH
2016 College of Veterinary Medicine Research Day, The Ohio State University in Columbus OH

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report

4.Impact

What was the impact on the development of the principle discipline of the project?

DKK1 was previously considered as the new therapeutic target for inhibiting the osteoblastic phenotype of bone-metastatic cancers [14]. Unexpectedly, our previous study found that Dkk1 did not only decrease osteoblastogenesis but also increased tumor growth in vivo [6]. In this study, we found evidence that Dkk1 may increase tumor growth by inducing the non-canonical Wnt/JNK signaling pathway. Inhibiting the non-canonical Wnt/JNK pathway using SP600126 resulted in significantly decreased tumor volumes. This finding improved our understanding of the role of Dkk1 in prostate cancer as well as improving a potential new therapeutic strategy for patients with prostate cancer.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

SP600125 could be one of the useful treatments for prostate cancer patients with high levels of Dkk1

What was the impact on society beyond sciences and technology?

The results of this research would help improve the quality of life of patients with prostate cancer.

5.Changes/Problems

Change in approach and reasons for change

Nothing to Report

Actual or anticipated problem or delay and action plans to solve them

Nothing to Report

Change that had significant impact on expenditures

Nothing to Report

Significant change in use or care of human subjects, vertebrate animals, biohazards, and or select agents

No change

6. Products

Publications, conference paper, and

presentations Journal publication:

Supsavhad W, Simmons JK, Dirksen WP, Elshafae SM, Hassan BB, Kohart NA, Altstadt LA, Demirer AA, Rosol TJ. Wnt signaling in prostate cancer growth and bone metastasis. *The Prostate*, in preparation for publication.

Book or other non-periodical, one-time publications: Nothing to Report

Other publications, conference papers, and presentations:

Wnt Signaling in Prostate Cancer Bone Metastasis, Jessica Simmons, Wessel Dirksen, Thomas Rosol, The Ohio State University, Columbus, OH, USA (Poster presentation at the 2014 Annual Meeting of the Society for Toxicologic Pathologists in Washington DC.)

Wnt signaling in prostate cancer growth and bone metastases, Wachiraphan Supsavhad, Jessica K. Simmons, Wessel P. Dirksen, Said M. Elshafae, Bardes B. Hassan, Nicole A. Kohart, Lucas A. Altstadt, Aylin A. Demirer and Thomas J. Rosol, Department of Veterinary Biosciences, The Ohio State University (Poster presentation at 2016 College of Veterinary Medicine research day in Ohio)

Website(s) or other internet site(s): Nothing to Report

Technologies or techniques: Nothing to Report

Inventions, patent applications, and/or licenses: Nothing to Report

Other products: Nothing to Report

7. Participants & other collaborating organizations

What individuals have worked on the project?

PI has been changed from Dr. Jessica Simmons to Dr. Wachiraphan Supsavhad since April 1st 2015.

Name:	Wachiraphan Supsavhad, DVM, MS
Project Role:	PhD Graduate Student
Research ID:	0000-0001-6462-5139
Nearest person month	18 months
Contribution to project:	Provides majority of laboratory work for the project. Mentored by Dr. Rosol.
Funding support:	Nothing in addition to the DOD fellowship.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to Report

8. Special reporting requirements

Collaborative awards

Nothing to report

Quad chart

Nothing to report

9. Appendices

Poster abstract; 33rd annual symposium Society of Toxicologic Pathology

Wnt Signaling in Prostate Cancer Bone Metastasis by Jessica Simmons, Wessel Dirksen, Thomas Rosol. The Ohio State University, Columbus, OH, USA

Poster abstract; 2016 CVM research day, The Ohio State University

Wnt signaling in prostate cancer growth and bone metastases, Wachiraphan Supsavhad, Jessica K. Simmons, Wessel P. Dirksen, Said M. Elshafae, Bardes B. Hassan, Nicole A. Kohart, Lucas A. Altstadt, Aylin A. Demirer and Thomas J. Rosol, Department of Veterinary Biosciences

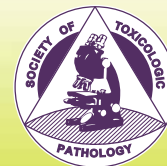
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TRANSLATIONAL PATHOLOGY:
Relevance of Toxicologic Pathology to Human Health

Poster Presentations



Society of Toxicologic Pathology

Poster Times and Poster Setup

Poster Setup

Sunday, June 22.....8:00 AM–3:00 PM
Your poster must be set up by 3:00 PM on Sunday, June 22.

Poster Presentation Times

(Please plan to attend your posters during the following times)

Sunday, June 22 (Welcome Reception)..... 6:00 PM–6:30 PM (Optional)
Monday, June 23..... 10:30 AM–11:00 AM and 3:00 PM–3:35 PM
Tuesday, June 24..... 9:45 AM–10:20 AM and 3:00 PM–3:35 PM
Wednesday, June 25..... 10:05 AM–10:40 AM

Poster Teardown

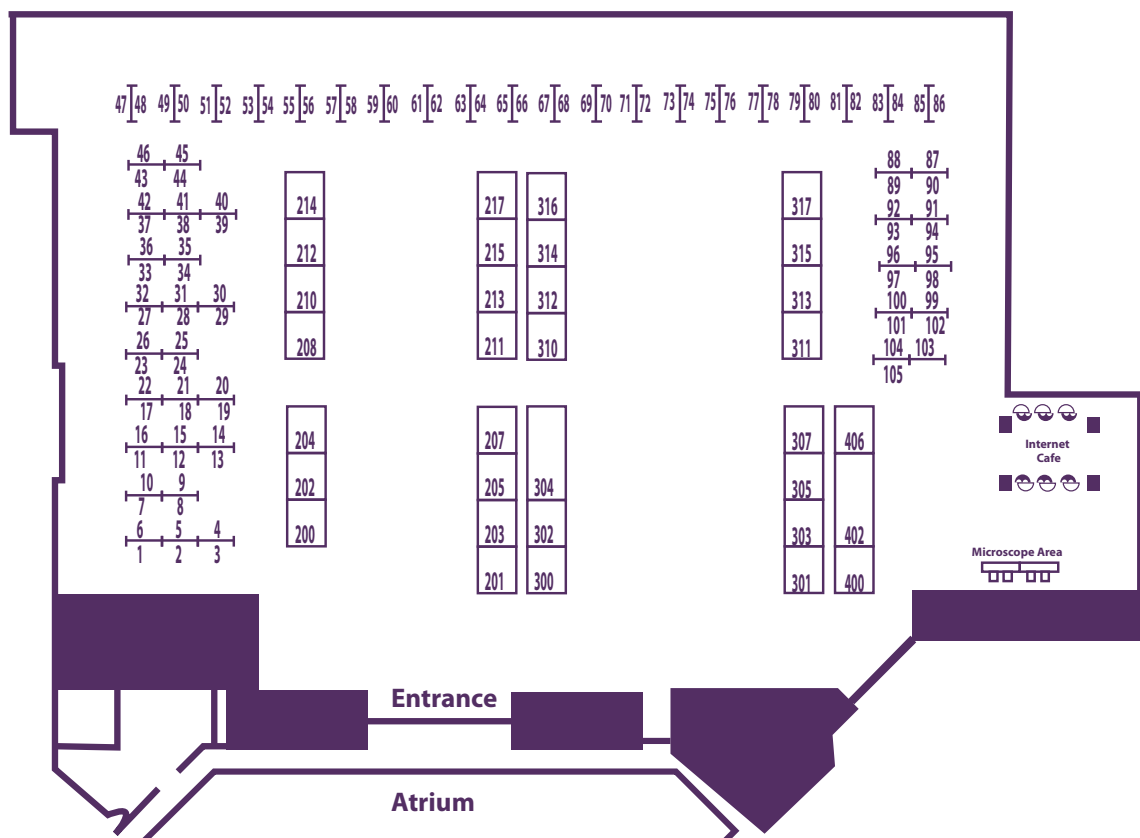
Wednesday, June 25..... 11:30 AM–1:00 PM
If your poster is not removed before 1:00 pm on Wednesday, June 25, it will be removed and placed near the Registration Desk for pickup.

Young Investigator Judging Times

Monday, June 23..... 7:15 AM–8:00 AM, 10:30 AM–11:00 AM, and 3:00 PM–3:35 PM
Tuesday, June 24..... 9:45 AM–10:20 AM

Marriott Wardman Park Hotel—Exhibit Hall C

Booths, Posters, Internet Café, Microscope and Digital Slide Viewing Area



Poster Presentations



Poster Presentations

33rd Annual Symposium

Washington, DC

Marriott Wardman Park Hotel

June 22–26, 2014

Poster Presentation Index

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Scan Me!

Poster Categories:

Young Investigator Candidates 1–20

Biomarkers 21–26

General Pathology/Toxicologic Pathology 27–52

New Technologies 53–66

Oncology/Carcinogenesis 67–81

Systemic/Organ-Specific Toxicologic Pathology 82–104

§ Student Travel Award Winners

1 Protective Role of Sildenafil Against Carbon Tetrachloride-Induced Nephrotoxicity by Augmenting the Availability of Nitric Oxide and Antioxidant Enzymes

Shubham Goyal¹, Vaneeta Rani², Sawati Sharma¹, Nitin Verma¹, ¹School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences and Technology, Vill-Makhnumajra, Baddi, Distt. Solan, HP, India, ²Himalyan Institute of Pharmacy, Kala-Amb, Distt. Sirmour, HP, India

2 Effects of Rosiglitazone on β -Cell Function in Metabolic Syndrome Patients with Impaired Glucose Tolerance

Ravinesh Mishra¹, Anees A Siddiqui², Asif Husain², Mohd Rashid¹, Viny Srinivasan³, ¹School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences and Technology, Makhnumajra, Baddi, Distt. Solan, HP, India, ²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi, India, ³Department of US Safety Evaluation, L'Oréal, Clark, NJ, USA

§3 Comparative Toxicity and Efficacy of Engineered Anthrax Lethal Toxin Variants with Broad Anti-Tumor Activities

Diane Peters^{1,2}, Benjamin Hoover³, Loretta Grey Cloud¹, Shihui Liu³, Alfredo Molinolo¹, Stephen Leppla³, Thomas Bugge¹, ¹National Institute of Dental and Craniofacial Research, Bethesda, MD, USA, ²Tufts University Sackler School of Graduate Biomedical Sciences, Boston, MA, USA, ³National Institute of Allergy and Infectious Disease, Bethesda, MD, USA

4 Reproductive Staging in the Göttingen Miniature Pig

Rebecca Terry¹, Jan Klapwijk², Franck Chanut², ¹Royal Veterinary College, London, UK, ²GlaxoSmithKline, Ware, UK

5 Histologic Changes Following Acid Reflux Challenge in Porcine Vocal Folds

Abigail Durkes¹, Paul Snyder¹, Preeti Sivasankar¹, ¹Purdue University, West Lafayette, IN, USA

6 Subchronic Toxicological Study of Two Artemisinin Derivatives in Dogs

Ji-ye Yin¹, He-mei Wang¹, Quan-jun Wang¹, Ri-gao Ding¹, ¹Beijing Institute of Pharmacology and Toxicology, Beijing, China

7 A Maximum Tolerated and Therapeutic Dose of Cholic Acid Rescues the Lethal Effects of Ethanol in the Zebrafish (*Danio rerio*) Fetal Alcohol Model: A Toxicity, Tolerability, and Macroscopic Morphological Study

Shemikah Colleton¹, Curtis Colleton², James Marrs⁴, Christian Lawrence³, Courtney Curtis⁴, Kara Maloney³, Mariah Gardner¹, Madeleine Walsh¹, Philip Marx², Lora Becker¹, ¹University of Evansville, Evansville, IN, USA, ²Bristol-Myers Squibb Company, Mt. Vernon, IN, USA, ³Boston Children's Hospital Aquatic Resources, Boston, MA, USA, ⁴Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA

8 Chronic Comparative Embryonic Tolerability, Toxicity, and Macroscopic Morphological Study of Sub-Lethal Rectified and Medical Grade Ethanol Exposure in Zebrafish (*Danio rerio*)

Shemikah Colleton¹, Curtis Colleton², James Marrs⁴, Christian Lawrence³, Courtney Curtis⁴, Kara Maloney³, Madeleine Walsh¹, Mariah Gardner¹, Philip Marx², Lora Becker¹, ¹University of Evansville, Evansville, IN, USA, ²Bristol-Myers Squibb Company, Mt. Vernon, IN, USA, ³Boston Children's Hospital Aquatic Resources Program, Boston, MA, USA, ⁴Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA

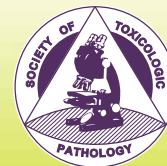
§9 Perfusion Recovery in a Mouse Model of Hind Limb Ischemia Is Enhanced by Mesenchymal Stem Cell-Laden Alginate Implants

Artem Shkumatov¹, Min Kyung Lee¹, Hyun Joon Kong¹, ¹University of Illinois, Urbana-Champaign, IL, USA



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10 Development of a Tissue Image Analysis Algorithm for Celiac Drug Development

Erik Hagendorn¹, Christa Whitney-Miller², G. David Young¹, Steve Potts¹, ¹Flagship Biosciences, Boulder, CO, USA, ²University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

§11 Wnt Signaling in Prostate Cancer Bone Metastasis

Jessica Simmons¹, Wessel Dirksen¹, Thomas Rosol¹, ¹The Ohio State University, Columbus, OH, USA

§12 Elucidating the Carcinogenic Mode of Action of Diuron on Rat Urothelium

Mitscheli Da Rocha^{1,2}, Lora Arnold², Puttappa Dodmane², Maria Luiza de Oliveira¹, Ana Paula Cardoso¹, Merielen Pontes¹, Karen Pennington², David Muirhead², Fang Qiu², Samuel Cohen², João Lauro de Camargo², ¹São Paulo State University, Botucatu, Brazil, ²University of Nebraska Medical Center, Omaha, NE, USA

§13 ALDH1B1 Is Crucial for Colon Tumorigenesis by Modulating Wnt/ β -catenin, Notch and PI3K/Akt Signaling Pathways

Surendra Singh¹, John Arcaroli², Ying Chen¹, David Orlicky⁴, David Thompson³, Wells Messersmith², Vasilis Vasilou¹, ¹Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, ²Division of Medical Oncology, University of Colorado School of Medicine, Aurora, CO, USA, ³Department of Clinical Pharmacy, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, ⁴Department of Pathology, University of Colorado School of Medicine, Aurora, CO, USA

14 Cigarette Smoke Condensate Induces Early Epithelial-Mesenchymal Transition (EMT) in Cultured Human Ectocervical Cells

Xiaohua Gao¹, Linda Yu¹, Lysandra Castro¹, Deloris Sutton¹, Connie Cummings¹, Daniel Morgan¹, Grace Kissling², Darlene Dixon¹, ¹NIEHS, NTP, Research Triangle Park, NC, USA, ²NIEHS, Research Triangle Park, NC, USA

15 Identification of Differentially Expressed Genes Defining Heterogeneity of Cancer Cells by RNA-Seq using Next Generation Sequencing

Yongbaek Kim^{1,2}, Myung-Chul Kim¹, Na-Yon Kim¹, Hang-A Kim¹, Cui-Feng Ji¹, ¹Laboratory of Clinical Pathology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea, ²Research Institution of Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

16 An Allelic Variant of the Mechanistic Target of Rapamycin (mTOR) Leads to Altered DNA Damage Repair

Joy Gary^{1,2}, Shuling Zhang¹, Ke Zhang¹, Wendy Dubois¹, Aleksandra Michalowski¹, Beverly Mock¹, ¹CCR, NCI, NIH, Bethesda, MD, USA, ²Michigan State University, East Lansing, MI, USA

17 Possible Mechanisms Underlying Exacerbation of Osmotic Nephrosis Caused by Pre-Existing Kidney Injury

Kohei Matsushita¹, Shinji Takasu¹, Yuji Ishii¹, Ken Kuroda¹, Aki Kijima¹, Keisuke Kitaura², Makoto Sato², Satoshi Matsumoto², Kumiko Ogawa¹, Takashi Umemura¹, ¹Division of Pathology, National Institute of Health Sciences, Tokyo, Japan, ²Safety Research Center, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan

18 Role of Cysteine-Rich Secretory Protein LCCL Domain Containing 2 [CRISPLD2] in Bile Duct Epithelial Branching Morphogenesis during Hepatic Fibrosis and Cholangiocarcinoma Progression

Chandrasagar Saravanan¹, Cheryl Spence¹, James Trevaskis¹, Xiaosong Wang¹, Jean-Rene Galarneau¹, William Chutkow¹, Keith Mansfield¹, ¹Novartis Institutes for Biomedical Research, Cambridge, MA, USA

19 Macrophage-Derived Galectin-3 Is the Key Regulator of Acute Hepatic Fibrogenesis in Rats

Hossain M. Golbar¹, Takeshi Izawa¹, Bondoc Alexandra¹, Kavindra K. Wijesundera¹, Anusha H. Tennakoon¹, Chisa Katou-Ichikawa¹, Miyuu Tanaka¹, Mitsuru Kuwamura¹, Jyoji Yamate¹, ¹Osaka Prefecture University, Izumisano City, Osaka, Japan

§20 Improved LV Function in Levosimendan-Treated Rats with Reversed Volume Overload Heart Failure Correlates with Normalized Alpha-to-Beta Myosin Heavy Chain Expression

Kristin Wilson^{1,2}, Mary Cismowski^{1,2}, Pamela Lucchesi^{1,2}, ¹The Ohio State University, Columbus, OH, USA, ²Nationwide Children's Hospital, Columbus, OH, USA

21 The Combined Use of Structural and Functional Cardiac Biomarkers Enhances the Ability to Understand the Pathogenesis of Cardiotoxicity

William Reagan¹, Vincent Bernardo¹, Raju Mantena³, Bernie Buetow², Jianying Wang², Allison Vitsky², Hugh Barton⁴, Jon Heyen², Karen Leach⁵, Dingzhou Li¹, Carrie Northcott¹, David Potter¹, Deb Burt¹, Rick Goldstein¹, Wendy Hu², Nick Edmunds¹, ¹Pfizer Drug Safety Research and Development, Groton, CT, USA, ²Pfizer Drug Safety Research and Development, La Jolla, CA, USA, ³Pfizer Drug Safety Research and Development, Cambridge, MA, USA, ⁴Pfizer Pharmacokinetics Dynamics and Metabolism, Groton, CT, USA, ⁵Pfizer Compound Safety Prediction Group, Groton, CT, USA



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22 Determination of Atrial Natriuretic Peptide in Sprague-Dawley Rat and C57BL/6 Mouse Serum: Comparison Between Two Immunoassays

Ahmad Farhad¹, Mehrdad Ameri¹, Jim Turk¹, ¹Comparative Biology and Safety Sciences, Amgen, Inc., Thousand Oaks, CA, USA

23 Altered Ubiquitin Expression in the Airways of Diacetyl-Exposed Mice

Ann Hubbs¹, Kara Fluharty¹, Rebekah Edwards¹, John Grantham^{1,2}, Linda Sargent¹, Steven Reynolds¹, Robert Mercer¹, Michael Kashon¹, Lori Battelli¹, Mark Jackson¹, Amy Cumpston¹, Travis Goldsmith¹, David Frazer¹, Tiffani Munro¹, Winnie Moyers¹, Kimberly McKinstry¹, Sherri Friend¹, Krishnan Sriram¹, ¹National Institute for Occupational Safety and Health, Morgantown, WV, USA, ²West Virginia University, Morgantown, WV, USA

24 Biomarkers of Hypercoagulability in a Rat Sepsis-Induced Model of Non-Overt Disseminated Intravascular Coagulation (DIC)

MB Brooks¹, JR Turk², CE Fishman³, DW Wilson⁴, AE Schultze⁵, JB Pierson⁶, members of the Cardiac Biomarkers Working Group of the HESI Cardiac Safety Technical Committee, ¹Cornell University, Ithaca, NY, USA, ²Amgen, Inc., Thousand Oaks, CA, USA, ³GlaxoSmithKline, King of Prussia, PA, USA, ⁴University of California, Davis, Davis, CA, USA, ⁵Eli Lilly and Company, Indianapolis, IN, USA, ⁶HESI, Washington, DC, USA

25 Application of Predictive Biomarkers for Renal Toxicity in Drug Development

Kaoru Toyosawa¹, Takayuki Iwaisako¹, Akiko Kusayanagi¹, Izumi Matsumoto¹, Mami Kouchi¹, Tomoaki Tochitani¹, Toru Yamada¹, Hitoshi Funabashi¹, ¹Dainippon Sumitomo Pharma Co., Ltd., Osaka, Osaka, Japan

26 Investigation of Urine Biomarker Performance in Rat Glomerular Injury Models

Bruce Homer¹, Steven Bailey², Eva Nagiec¹, Deborah Burt³, Mira Ko⁴, Karen Percival², Rounak Nassirpour², Radi Zaher², James Warneke², Thomas Brown³, ¹Pfizer, Inc., Cambridge, MA, USA, ²Pfizer, Inc., Andover, MA, USA, ³Pfizer, Inc., Groton, CT, USA, ⁴Pfizer, Inc., La Jolla, CA, USA

27 Unexpected Pituitary Pathology after Chronic Administration of MEDI412, a High Potency Anti-IgE Antibody

Mary McFarlane², William Iverson¹, Juerg Bluemel¹, ¹MedImmune, LLC, Gaithersburg, MD, USA, ²MedImmune, LLC, Cambridge, UK

28 Toxicological Findings in Beagle Dogs after 13 Weeks Oral Gavage of a DPP-4 Inhibitor

Jingjun Sun¹, Henglei Lu¹, Jianhong Pan¹, Junwen Qiao¹, Rongrong Tan¹, Hua Sheng¹, Jin Ren¹, ¹Center for Drug Safety Evaluation and Research, Shanghai Institute of Materia Medica, Shanghai, China

29 Dysregulation of Iron Homeostasis during Progression of Thioacetamide-Induced Liver Cirrhosis in Rats

Takeshi Izawa¹, Hiroshi Murakami¹, Rena Miyagi¹, Kavindra Wijesundera¹, Anusha Tennakoon¹, Golbar Hossain¹, Mitsuru Kuwamura¹, Jyoji Yamate¹, ¹Osaka Prefecture University, Osaka, Japan

30 Congenital Intrathoracic Left Kidney in a Cynomolgus Monkey

Henglei Lu¹, Jingjun Sun¹, Rongrong Tan¹, Jin Ren¹, ¹Center for Drug Safety Evaluation and Research, Shanghai Institute of Materia Medica, Shanghai, China

31 Repeated Subcutaneous Dose Toxicity Studies of Iron Oxide-Zinc Oxide Core-Shell Nanoparticles in C57BL/6 Mice

Jeong-Hwan Che¹, Jung-Hee Yoon¹, Byeong-Cheol Kang¹, Nam-Hyuk Cho², Seung Hyeok Seok², Young Keun Kim³, ¹Biomedical Research Institute, Seoul National University Hospital, Seoul, Republic of Korea, ²Department of Microbiology and Immunology, Seoul National University College of Medicine, Seoul, Republic of Korea, ³Department of Materials Science and Engineering and Pioneer Research Center for Biomedical Nanocrystals, Korea University, Seoul, Republic of Korea

32 Anti-Apoptotic Potential of Herbal Plant Extract in Rats with Cardiomyopathy

Harbir Kaur^{1,2}, Gurpreet Singh², Maninder Singh², Ankur Bansal², ¹School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences and Technology, Makhnumajra, Baddi, Distt. Solan, HP, India, ²Department of Pharmaceutics, Rayat Institute of Pharmacy, Railmajra, Distt. SBS Nagar, Punjab, India, ³Ranbaxy Laboratories, Ltd., Mohali, India

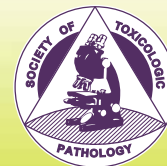
33 Artfactual Positive Urine Reagent Test Strip Reactions Caused by Common Contaminants in Laboratory-Housed Nonhuman Primates and Beagle Dogs

William Siska¹, Dennis Meyer¹, A. Eric Schultze², Catherine Brandoff¹, Jamie West¹, Steven Wirth¹, ¹Charles River Laboratories, Reno, NV, USA, ²Department of Pathology, Lilly Research Laboratories, Indianapolis, IN, USA



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34 Short-Term Carcinogenic Screening Study Using a Limited Number of Tg-rasH2 Mice

Yutaka Nakanishi¹, Satoshi Tsuji¹, Masaki Wakamatsu¹, Hironori Takagi¹, Minoru Sasaki¹, Shunsuke Tsutsumi¹, Yasushi Sato¹,
¹Drug Safety and Pharmacokinetics Laboratories, Taisho Pharmaceutical Co., Ltd., Saitama, Japan

35 Comparison of Historical Control Parameters Derived from Subchronic Studies Conducted in CD® IGS Rats Fed 5002 or 5CR4 Certified Diets

Mark Morse¹, Binod Jacob¹, Aaron Sargeant¹, ¹Charles River Laboratories, Spencerville, OH, USA

§36 Minocycline, a Putative Neuroprotectant, Co-Administered with Doxorubicin-Cyclophosphamide Chemotherapy in a Xenograft Model of Triple-Negative Breast Cancer

Lauren Himmel¹, Maryam Lustberg¹, A. Courtney DeVries¹, Charles Shapiro¹, Ching-Shih Chen¹, Samuel Kulp¹, ¹The Ohio State University, Columbus, OH, USA

37 Conditioning Agents for Gene Therapy: Busulfan vs. Irradiation—Common Histopathology Findings in Mice Used in Preclinical Studies.

Franck Chanut¹, Francesca Sanvito³, Giuliana Ferrari², Patrizia Cristofori¹, ¹GlaxoSmithKline David Jack Centre for R&D, Ware, UK, ²HSR-TIGET Ospedale San Raffaele, Milan, Italy, ³Pathology Unit, San Raffaele Scientific Institute, Milan, Italy

38 Sex Differences and Litter-Based Effects on Hematologic Parameters from 28-Day Old Harlan Sprague Dawley (Hsd:Sprague Dawley SD) Rats

Michelle Cora¹, Greg Travlos¹, Grace Kissling¹, Matt Stout¹, Angela King-Herbert¹, Molly Vallant¹, Chad Blystone¹, ¹National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

39 Common Bile Duct Injury in Wistar Rats Induced by Sorafenib Tosylate

Ke Chen^{1,2}, Yuanyuan Deng^{1,2}, Fen Wang^{1,2}, Hongxia Li^{1,2}, Xiaobo Cen^{1,2}, Chunyan Hu^{1,2}, Peter Mann³, ¹National Chengdu Center for Safety Evaluation, West China Hospital, Chengdu, Sichuan, China, ²West China Frontier Pharma Tech Co., Ltd., Chengdu, Sichuan, China, ³EPL Northwest, Seattle, WA, USA

40 Clinical Pathology Reference Intervals for Healthy Göttingen Minipigs Based on Tolerance Intervals

Hala Willis¹, Jodi Boysza¹, Alex Rodriguez¹, Vicky Satterfield¹, Carie Kimbrough¹, Holly Jordan¹, ¹GlaxoSmithKline, Research Triangle Park, NC, USA

41 Subchronic Inhalation Exposure of Rats to Libby Amphibole and Amosite Asbestos: Effects at 18 Months Post Exposure

Gabrielle A. Willson¹, Darol E. Dodd², Kay Roberts², Henry G. Wall¹, Stephen H. Gavett³, ¹Experimental Pathology Laboratories, Inc., Research Triangle Park, NC, USA, ²The Hamner Institutes for Health Sciences, Research Triangle Park, NC, USA, ³US EPA, Research Triangle Park, NC, USA

42 A 21st Century Roadmap for Human Health Risk Assessment

Douglas Wolf¹, Ammie Bachmann², Alan Boobis³, Samuel Cohen⁴, Michael Dellarco⁵, Ian Dewhurst⁶, John Doe⁷, Nancy Doerrer⁸, Angelo Moretto⁹, Timothy Pastoor¹, Richard Phillips², J. Craig Rowlands¹⁰, Jennifer Tanir⁸, Michelle Embry⁸, ¹Syngenta, Ltd., Greensboro, NC, USA, ²ExxonMobil Biomedical Sciences, Inc., Annandale, NJ, USA, ³Imperial College London, London, UK, ⁴University of Nebraska Medical Center, Omaha, NE, USA, ⁵National Institute of Child Health and Human Development, Bethesda, MD, USA, ⁶UK Chemicals Regulation Directorate, York, UK, ⁷Parker Doe Partnership LLP, Chesire, UK, ⁸ILSI Health and Environmental Sciences Institute, Washington, DC, USA, ⁹University of Milan, Milan, Italy, ¹⁰The Dow Chemical Company, Midland, MI, USA

43 Lack of In Vivo Genotoxicity and Rat Toxicity of Myricitrin Administered at Dietary Levels up to 5%

Shim-mo Hayashi¹, Jeffrey Davis², Cheryl Hobbs², Carol Swartz², Leslie Recio², Robert Maronpot³, ¹San-Ei Gen FFI, Inc., Osaka, Japan, ²Integrated Laboratory Systems, Inc., Research Triangle Park, NC, USA, ³Maronpot Consulting, LLC., Raleigh, NC, USA

44 Development of Photoaging Model Using UVB and Heat

Il-Hong Bae¹, Ji-Hae Lee¹, Dae-Yong Kim¹, ¹College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

45 A Decade of Non-Neoplastic Histologic Background Lesions in Göttingen Minipigs at MPI Research

Charlotte Hollinger¹, Keith Nelson^{2,1}, ¹Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA, ²MPI Research, Mattawan, MI, USA



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46 Isoproterenol Induced Skeletal Troponin Elevation in Sprague Dawley Rats

Malar Pannirselvam¹, Quincey Simmons¹, Jessica Wan¹, Suzanne Botts¹, David Brewster¹, ¹Vertex Pharmaceuticals, Boston, MA, USA

§47 Effects of Perinatal Exposure to Single or Mixed Fungicides on the Female Rat Reproductive System

Viviane Pascotto¹, Carla Franchi¹, Janete Franci², Ruither Carolino¹, João Lauro Camargo¹, ¹São Paulo State University UNESP Medical School, Botucatu, Brazil, ²School of Dentistry of Ribeirão Preto, University of São Paulo, USP, Ribeirão Preto, Brazil

48 Lysosomal Drug Accumulation and Precipitation in Rats Seen with an Amphoteric Compound

Barbara Lenz¹, Urs Niederhauser¹, Holger Fischer¹, Rodolfo Gasser¹, Claus Riemer¹, Alexander Flohr¹, Thomas Weiser¹, Thomas Singer¹, Nicole Wytenbach¹, Mudher Albassam¹, Monira Siam¹, ¹Hoffmann-La Roche, Basel, Switzerland

49 Mitochondrial Alteration in CD1 Mice Associated with Prenatal Exposures to Low Doses of Perfluorooctanoic Acid (PFOA): A PPAR α -Independent Mode of Action?

Erin Quist^{1,2}, Adam Filgo^{1,3}, Connie Cummings⁵, Grace Kissling⁴, Mark Hoenerhoff⁶, Suzanne Fenton¹, ¹NTP Laboratory Branch, NTP, NIEHS, NIH, DHHS, Research Triangle Park, NC, USA, ²Comparative Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA, ³Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC, USA, ⁴Biostatistics Branch, DIR, NIEHS, NIH, DHHS, Research Triangle Park, NC, USA, ⁵UltraPath Imaging, Durham, NC, USA, ⁶Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI, USA

50 Histologic Findings Associated with Chronic Femoral Artery Implantation of a Miniature Telemetry Blood Pressure Transmitter in Cynomolgus Monkeys

William Meier¹, Laura Kreckler¹, John Kremer¹, Mike Foley¹, Mark Holbrook², Niraj Tripathi¹, Alok Sharma¹, Emily Meseck¹, ¹Covance Laboratories, Madison, WI, USA, ²Covance Laboratories, Harrogate, UK

51 Non-Lesions, Misdiagnoses, Missed Diagnoses, and Other Interpretive Challenges in Fish Histopathology Studies: A Guide for Investigators, Authors, Reviewers, and Readers

Jeffrey Wolf¹, Wes Baumgartner², Vicki Blazer³, Alvin Camus⁴, Jeffery Engelhardt⁵, John Fournie⁶, Salvatore Frasca⁷, David Groman⁸, Michael Kent⁹, Lester Khoo¹⁰, Jerry Law¹¹, Eric Lombardini¹², Christine Ruehl-Fehlert¹³, Helmut Segner¹⁴, Stephen Smith¹⁵, Jan Spitsbergen¹⁶, Klaus Weber¹⁷, Marilyn Wolfe¹⁸, ¹Experimental Pathology Laboratories, Inc., Sterling, VA, USA, ²Department of Pathobiology/Population Medicine, College of Veterinary Medicine, Mississippi State, Mississippi State, MS, USA, ³US Geological Survey, Kearneysville, WV, USA, ⁴Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA, ⁵Experimental Pathology Laboratories, Inc., Camarillo, CA, USA, ⁶US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL, USA, ⁷Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT, USA, ⁸Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, ⁹Departments of Microbiology and Biomedical Sciences, Oregon State University, Corvallis, OR, USA, ¹⁰Mississippi State University, College of Veterinary Medicine, Stoneville, MS, USA, ¹¹Aquatic Ecotoxicology, North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA, ¹²Divisions of Comparative Pathology and Veterinary Medical Research, Department of Veterinary Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, ¹³Bayer HealthCare AG, Wuppertal, Germany, ¹⁴Centre for Fish and Wildlife Health, University of Bern, Bern, Switzerland, ¹⁵Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA, ¹⁶Fish Disease Research Group, Department of Microbiology, Oregon State University, Corvallis, OR, USA, ¹⁷AnaPath GmbH, Oberbuchsitzen, Switzerland, ¹⁸EPL, Inc., Sterling, VA, USA

52 Location Matters—the Behavior of Human Retinoblastoma Cells is Dependent on the Site of Implantation

Julia Baker¹, Shawna Jackman², ¹Charles River Pathology Associates, Frederick, MD, USA, ²Charles River Preclinical Services, Horsham, PA, USA

53 Quantitative Evaluation of Drug-Induced Microvascular Constriction in Mice Kidney Using a Novel Tool for 3D Geometrical Analysis of Ex Vivo Organ Vasculature

Raul Brauner², Yuval Ramot¹, Kongbin Kang^{2,4}, John V. Heymach³, Stacia Furtado^{2,4}, Abraham Nyska⁵, ¹Hadassah – Hebrew University Medical Center, Jerusalem, Israel, ²Bio-Tree Systems Inc., Framingham, MA, USA, ³MD Anderson Cancer Center, Houston, TX, USA, ⁴Brown University, Providence, RI, USA, ⁵Toxicologic Pathologist, Timrat, Israel

54 Significant Lessening of Local Reactions Following Continuous SC Administration of ND0701, a New Apomorphine Formulation for Parkinson's Disease—MRI and Histopathology Studies

Ronit Shaltiel-Karyo¹, Hanita Ovadia¹, Yonit Tzarfaty¹, Yael Schiffenbauer², Abraham Nyska³, Oron Yacoby-Zeevi¹, ¹NeuroDerm company, Ness-Ziona, Israel, ²Aspect Imaging, Shoham, Israel, ³Consultant in Toxicologic Pathology, and Tel Aviv University, Timrat, Israel



55 The Development of Image Analysis Algorithms to Objectively Characterize the Host Tissue Response to an Encapsulated Stem Cell Product

John J. O'Neil¹, Allison Rubin², Irina Batushansky², David Young³, ¹Janssen Research and Development, LLC., Raritan, NJ, USA, ²Janssen Research and Development, LLC., Springhouse, PA, USA, ³Flagship Biosciences, LLC., Boulder, CO, USA

56 The Use of Immunohistochemistry and Image Analysis to Objectively Characterize the Composition of a Stem Cell-Derived Therapy in Preclinical Studies

Jing Ying Ma¹, Allison Rubin², Irina Batushansky², John J O'Neil³, ¹Janssen Research and Development, LLC., La Jolla, CA, USA, ²Janssen Research and Development, LLC., Springhouse, PA, USA, ³Janssen Research and Development, LLC., Raritan, NJ, USA

57 First Time in Man Enabling Study Using Gene Therapy to Treat Beta-Thalassemia: Focus on the Histopathology

Franck Chanut¹, Francesca Sanvito^{3,2}, Francesca Tiboni², Giacomo Mandelli², Maria-Rosa Lidonnici², Paola Albertini², Luigi Naldini², Giuliana Ferrari², Patrizia Cristofori¹, ¹GlaxoSmithKline David Jack Centre for R&D, Ware, UK, ²San Raffaele-Telethon Institute for Gene Therapy (TIGET), Milan, Italy, ³Pathology Unit, San Raffaele Scientific Institute, Milan, Italy

58 Development of Tissue Image Analysis Tools to Identify Murine Pancreatic Intraepithelial Neoplasia

Famke Aeffner¹, Mirza Peljto¹, Michael O. Ports², Thorsten Hagemann³, G. David Young¹, ¹Flagship Biosciences, LLC., Boulder, CO, USA, ²Gilead Sciences, Seattle, WA, USA, ³Barts Cancer Institute, Queen Mary University of London, London, UK

§59 Photodynamic Therapy (PDT) Applied to the Primary Ehrlich Tumor Induces Inhibitory Effects on a Second Implant of the Same Tumor

Murilo Del-Grande¹, Maria Lucia Zaidan Dagli¹, ¹School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

60 Mass Spectrometry Imaging of Therapeutic Antibodies: Distribution of Unlabeled Trastuzumab in CB.17 SCID Mice Implanted with the Human Breast BT474 Xenograft

David BONNEL¹, Chassidy HALL², Robert J MULLIN² Kathryn Simon² and Jonathan STAUBER¹, ¹ImaBiotech, Lille, France
²Charles River Discovery Research Services-, North Carolina –Morrisville, USA

61 Mass Spectrometry Imaging in a Toxicology Study: Application in Induced Interstitial Pulmonary Fibrosis (IPF) Model

David Bonnel¹, Mary McElroy², Emeline Faloux¹, Gael Picard De Muller¹, Fabien Pamelard¹, Stephen Madden², Jonathan Stauber¹, ¹ImaBiotech, Lille, France, ²Charles River Discovery Research Services, Edinburgh, UK

62 Use of Legacy Data to Assess Species Concordance for Liver Injury

Stephanie Berry¹, Paul Bradley¹, ¹Instem Scientific, Cambridge, UK

63 Pathology of IL-33 Knock-In Mouse Model of Non-Resolving Inflammation

Maria Cristina De Vera Mudry¹, Juliana Bessa¹, Claas Aiko Meyer¹, Sonja Schlicht¹, Susan H. Smith², Thomas Weiser¹, Thomas Singer¹, Antonio Iglesias¹, Javier Cote-Sierra², ¹F. Hoffmann-La Roche Ltd., Basel, Switzerland, ²Stiefel, Research Triangle Park, NC, USA

64 Integration of MALDI Imaging and Optical Microscopy Allows New Insights into Molecular Distribution in Tissue

Katherine Kellersberger¹, M. Reid Groseclose², Stephen Castellino², ¹Bruker Daltonics, Billerica, MA, USA, ²GlaxoSmithKline, Research Triangle Park, NC, USA

65 A SEND Solution for Microscopic Pathology, In-Life, and Other Preclinical Toxicology Data Collected Using Multiple LIMS (Laboratory Information Management Systems)

Frederic Mura², Maro Schuster¹, Reto Aerni², Michael Wasko¹, Christina Wuermlin², Rich Buchanan¹, Amelia Bedoan², Laura Kaufman¹, ¹Preclinical Data Systems, Mt. Arlington, NJ, USA, ²Preclinical Data Systems, Basel, Switzerland

66 Characterizing and Diminishing Autofluorescence in Formalin-Fixed Paraffin-Embedded Human Respiratory Tissue

A. Sally Davis^{1,2}, Anke Richter³, Steven Becker¹, Jenna Moyer¹, Aline Sandouk¹, Jeff Skinner¹, Jeffery Taubenberger¹, ¹National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, ²North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA, ³Defense Resources Management Institute, School of International Studies, Naval Postgraduate School, Monterey, CA, USA



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68 Evaluation of Key Events in the Mode of Action for a Carry-Over Carcinogen in Mice

Charles Wood¹, April Lake^{1,2}, Greg Olson³, Michael George¹, Susan Hester¹, Anthony DeAngelo¹, ¹US Environmental Protection Agency, Research Triangle Park, NC, USA, ²University of North Carolina-Chapel Hill, Chapel Hill, NC, USA, ³Toxicologic Pathology Associates, Jefferson, AK, USA

69 Immunohistochemical Characterization of Carbon Nanotube-Induced Malignant Mesotheliomas in Rats

Susanne Rittinghausen¹, Anja Hackbarth¹, Heinrich Ernst¹, Uwe Heinrich¹, Albrecht Leonhardt², Dirk Schaudien¹, ¹Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany, ²Leibniz Institute for Solid State and Materials Research, Dresden, Germany

70 Proliferative Lesions in the Rete Ovarii of Aged Han Wistar Rats

Richard Haworth¹, Jaimini Kumar¹, David Lewis¹, ¹GlaxoSmithKline, Ware, UK

71 Early Detection of Carcinogenic Potential of CNT via Cell Proliferation Measurement

Dirk Schaudien^{1,3}, Anja Hackbarth¹, Heinrich Ernst¹, Albrecht Leonhardt², Uwe Heinrich¹, Susanne Rittinghausen¹, ¹Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany, ²Leibniz Institute for Solid State and Materials Research, Dresden, Germany

72 The RITA Database—The Value of Incidences of Tumors in Young Animals

Dirk Schaudien^{1,3}, Rupert Kellner^{1,3}, Matthias Rinke^{2,3}, ¹Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany, ²Bayer Pharma AG, Wuppertal, Germany, ³RITA-Registry of Industrial Toxicology Animal-Data, Hannover, Germany

73 Effect of Oral (Gavage) Administration of *Euphorbia tirucalli* (Aveloz) on the Ascitic Ehrlich Tumor Growth

Diego Pinha Alvez da Cruz¹, Gregory Mennecier¹, Andréia Latorre², Gokithi Akisue³, Mitsue Haraguchi⁴, Rueda Abu-Said⁵, Maria Lucia Zaidan Dagli¹, ¹University of São Paulo, São Paulo, Brazil, ²Adolpho Lutz Institute, São Paulo, Brazil, ³University of São Francisco, Bragança Paulista, Brazil, ⁴Biological Institute, São Paulo, Brazil, ⁵University of Santa Cruz, Ilhéus, Brazil

74 Immunohistochemical Characterization of ENU-Induced Brain Tumors in F344 Rats

Rebecca Moore¹, Holly Kolenda-Roberts², Nancy Harris¹, Young-Man Cho³, Kumiko Ogawa³, Jerry Hardisty¹, Rodney Miller¹, ¹Experimental Pathology Laboratories, Inc., Research Triangle Park, NC, USA, ²SNBL USA, Ltd., Everett, WA, USA, ³National Institute of Health Sciences, Tokyo, Japan

75 Influence of Processing Methods for Scanning Electron Microscopy in the Detection of Diuron-Induced Ultrastructural Alterations in the Rat Urinary Bladder

Rafaela Fava¹, Ana Paula Cardoso¹, Mitscheli da Rocha¹, Merielen Nascimento e Pontes¹, João Lauro de Camargo¹, Maria Luiza de Oliveira¹, ¹UNESP-São Paulo State University, Botucatu, Brazil

76 Role of Histone Citrullination in Macrophage Extracellular Trap (MET) Release and Characterization of METs in Human Tongue Squamous Cell Carcinoma

Sunish Mohanan^{1,2}, Angela Yan², Lynne Anguish², Dalton McLean², Sachi Horibata², Paul Thompson⁴, Neil A. Iyengar³, Andrew J. Dannenberg³, Scott A. Coonrod², ¹Eli Lilly and Company, Indianapolis, IN, USA, ²Baker Institute, Cornell University, Ithaca, NY, USA, ³Weill School of Medicine, Cornell University, New York, NY, USA, ⁴The Scripps Research Institute, Jupiter, FL, USA

77 NNK and Enantiomers of Its Metabolite, NNAL, Constituents of Tobacco Products, Induce Lung Tumors That Metastasize to Pancreas in F-344 Rats

Ramesh C. Kovi^{1,2}, Charles S. Johnson^{1,2}, Silvia Balbo², Stephen S. Hecht², M. Gerard O'Sullivan^{1,2}, ¹Comparative Pathology Shared Resource, St. Paul, MN, USA, ²Masoni Cancer Center, University of Minnesota, Twin Cities Campus, MN, USA

78 Hitherto Unknown Type of Adenocarcinoma in the Rat Mammary Gland

Heike Marxfeld¹, Silke Treumann¹, Karin Küttler¹, Sibylle Gröters¹, Bennard van Ravenzwaay¹, ¹BASF SE, Ludwigshafen, Germany

79 Epigenetic Regulation of Transcription Factor Promoter Regions by Low-Dose Genistein through MAPK and MSK1 Nongenomic Signaling

Linda Yu¹, Kyle Ham¹, Xiaohua Gao¹, Lysandra Castro¹, Norris Flagler¹, Ray Dong¹, Grace E. Kissling², Trevor K. Archer², Darlene Dixon¹, ¹NIEHS, NTP, Research Triangle Park, NC, USA, ²NIEHS, Research Triangle Park, NC, USA



80 Inhibitory Effects of Pequi (*Cariocar brasiliense* Camb) Oil on Preneoplastic Lesions in a Mouse Hepatocarcinogenesis Model

Simone Morais Palmeira¹, Paula Regina Pereira Silva¹, Cesar Kopi Grisoli², Paulo Hilário Nascimento Saldiva¹, Juliana Shimara Pires Ferrão¹, Maria Lucia Zaidan Dagli¹, Francisco Javier Hernandez-Blazquez¹, ¹University of São Paulo, São Paulo, Brazil, ²Federal University of Brasilia, Brasilia, Brazil

81 Multifunctional Photo-Theranostic Cancer Targeting Nanoporphyrin

Tzu-yin Lin¹, Yuanpei Li¹, Luo Yan¹, Qiangqiang Liu¹, Sebastian Wachsmann-Hogiu¹, Chongxian Pan¹, Kit Lam¹, ¹University of California-Davis, Sacramento, CA, USA

82 Post-Natal Development of the Ovary in the Rat

Catherine Picut¹, Amera Remick¹, Lydia Parker¹, Cynthia Swanson¹, Darlene Dixon², ¹WIL Research, LLC, Hillsborough, NC, USA, ²NIEHS, Research Triangle Park, NC, USA

83 Post-Natal Development of the Testes in the Rat

Catherine Picut¹, Cynthia Swanson¹, Lydia Parker¹, Amera Remick¹, ¹WIL Research, LLC, Hillsborough, NC, USA

84 The Estrous Cycle in Young Sexual Mature Göttingen Minipigs: A Morphologic Approach

Eveline de Rijk¹, Birgit Peter¹, Joost Lensen¹, Helle Lorentsen², Hetty van den Brink¹, ¹WIL Research Europe B.V., 's-Hertogenbosch, The Netherlands, ²Ellegaard Göttingen Minipig A/S, Dalmoose, Denmark

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Mehrdad Ameri¹, Ryan Morgan¹, Kim Stocking¹, Marnie Higgins-Garn¹, ¹Comparative Biology and Safety Sciences, Amgen Inc., Thousand Oaks, CA, USA

86 Wnt Inhibition Effects on Bone Formation in Preclinical Models Predicts Effects in Patients

Rene Meisner¹, Jie Wei¹, Jalpa Shah¹, Jennifer Cain¹, Tim Hoey¹, ¹OncoMed Pharmaceuticals, Redwood City, CA, USA

87 Ultrastructural Changes in Renal Papillary Duct Epithelium of Rats Treated with Trimethyltin Chloride

Kenichiro Kasahara¹, Hiroshi Edamoto¹, Mizuho Takagi¹, Ryo Ando¹, Kayoko Kudo¹, Rie Andoh¹, Kazutoshi Tamura¹, ¹BoZo Research Center, Inc., Shizuoka, Japan

88 Investigation of CNS Findings Following Raxibacumab Treatment against Inhalation Anthrax in the New Zealand White Rabbit Model

Maggie Dempster¹, Thi-Sau Migone², Sally Bolmer³, John Zhong⁴, Linda Hendey⁵, Dan Sanford⁵, Daphne Vasconcelos⁵, ¹GlaxoSmithKline, King of Prussia, PA, USA, ²Igenica, Burlingame, CA, USA, ³Consultant, San Francisco, CA, USA, ⁴Biogen Idec, Cambridge, MA, USA, ⁵Battelle, Columbus, OH, USA

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90 Immunohistochemistry on KIM-1 and BrdU Labeling of Cell Injury and Proliferation from 6-Month Repeated Dose Oral Toxicity Study of Canagliflozin in the Male Rat

Jing Ying Ma¹, Sandra De Jonghe², Jim Proctor³, Mark D. Johnson³, Rao N. V. S. Mamidi³, Calvert Loudon⁴, Sandra Snook¹, ¹Janssen R&D, LLC, La Jolla, CA, USA, ²Janssen R&D, LLC, Beerse, Belgium, ³Janssen R&D, LLC, Raritan, NJ, USA, ⁴Janssen R&D, LLC, Spring House, PA, USA

91 Interpretation of Rodent Respiratory Histopathology Following Repeat Inhalation Exposures to Agrochemical Aerosols with Irritant Properties

Ronnie Chamanza¹, Jayne A Wright¹, Pramila Singh², Paul Hinderliter², Douglas Wolf², Richard Lewis¹, ¹Syngenta, Jealott's Hill, Bracknell, Berkshire, UK, ²Syngenta, Greensboro, NC, USA

92 Female SDT Fatty Rat Shows Non-Alcoholic Steatohepatitis (NASH)-Like Hepatic Lesions

Katsuhiro Miyajima¹, Takeshi Ota², Yoshiaki Katsuda², Yukihito Ishii², Kochi Kakimoto¹, Yuzo Yasui¹, Yusuke Kemmochi¹, Akiko Anagawa-Nakamura¹, Kaoru Toyoda¹, Eriko Tanigai¹, Akemi Takahashi¹, Yoshifumi Miyakawa², Toshiyuki Shoda¹, ¹Japan Tobacco Inc. Central Pharmaceutical Research Institute, Hadano, Kanagawa, Japan, ²Japan Tobacco Inc. Central Pharmaceutical Research Institute, Takatsuki, Osaka, Japan



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94 Conduritol β -Epoxide-Induced Neuroinflammation in C57Bl/6J Mice: A Translational Drug Discovery Tool for Neuropathic Gaucher Disease

Dinesh Bangari¹, John Marshall¹, Errin Roberts¹, Amy Allaire¹, Eva Budman¹, John Leonard¹, Beth Thurberg¹, ¹Genzyme, Framingham, MA, USA

95 Normal Anatomy, Histology and Spontaneous Pathology of the Nasal Cavity of the Cynomolgus Monkey

Ronnie Chamanza^{1,2}, Ian Taylor³, Colin Hill³, Mark Swann³, Joel Goodchild³, Kane Goodchild³, Jane Schofield³, Mark Aldous³, Michela Gregori², Vasanthi Mowat², ¹Syngenta Limited, Jealott's Hill, Bracknell, Berkshire, UK, ²Huntingdon Life Sciences, Huntingdon, Cambridgeshire, UK, ³Huntingdon Life Sciences, Eye, Suffolk, UK

96 Background Incidence of Spontaneous Adrenal Gland Lesions of Control Charles River CD-1 Mice (CrI: CD-1(ICR) BR) Used in 104-Week Toxicity Studies

Claudio Petterino¹, Stuart Naylor¹, Sydney Mukaratirwa¹, Alys Bradley¹, ¹Charles River, Ltd., Trarant, UK

97 Mesenchymal Tissue Formation in Renal Tubule Lumen of Cyclosporine-Induced Acute Kidney Injury in a Cynomolgus Monkey

Kiyonori Kai¹, Satoko Sato¹, Kayoko Ishikawa¹, Takashi Yamaguchi¹, Munehiro Teranishi¹, Wataru Takasaki¹, ¹Daiichi Sankyo Co., Ltd., Tokyo, Japan

98 Exogenous Human Thioredoxin-1 Ameliorates Acetaminophen-Induced Acute Liver Toxicity and Liver Failure through Reducing Peroxynitrite and Inhibiting Degradation of Endogenous Thioredoxin-1

Byungwoo Lee¹, Jonghyeok Ko¹, Seonyong Kim¹, Byungil Yoon¹, ¹Kangwon National University, Chuncheon, Gangwon-do, Republic of Korea

99 Comparative Histology of Mouth Mucosae (Sublingual Region)

Catherine Thirion-Delalande¹, Cécile Fisch², Roy Forster¹, Bernard Palate¹, ¹CiToxLAB, Evreux, France, ²Stallergenes S.A., Antony, France

§100 Non-Neoplastic Histologic Cutaneous Background Lesions in Göttingen Minipigs and Findings at Treatment Control Sites

Charlotte Hollinger¹, Keith Nelson^{2,1}, ¹Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA, ²MPI Research, Mattawan, MI, USA

101 Temporal Patterns of Candidate Biomarkers of Vascular Injury in the IL2 Rat Model

Cristina Bertinetti-Lapatki¹, Natalie Keirstead², Denise Knapp¹, Igor Mikaelian³, James R. Turk⁴, Bradley E. Enerson⁵, Holly W. Smith⁶, ¹Hoffmann-La Roche, Inc., Basel, Switzerland, ²AstraZeneca, Waltham, MA, USA, ³Abbvie, Inc., Worcester, MA, USA, ⁴Amgen, Inc., Thousand Oaks, CA, USA, ⁵Pfizer, Inc., Groton, CT, USA, ⁶Eli Lilly, Indianapolis, IN, USA

102 Renal Epithelial Hyperplasia Caused by Urinary Crystals of a Novel GKA and Its Metabolites after Acute Dosing in Sprague Dawley Rats

ES Tien³, NE Everds², JR Turk¹, MP Nguyen², Q Ye⁴, P Cao⁴, L Jim⁴, KA Samoya⁴, PD Schnier⁴, RC Kelly⁴, JF Schroeder¹, CA Afshari¹, ¹Amgen, Inc., Thousand Oaks, CA, USA, ²Amgen, Inc., Seattle, WA, USA, ³Amgen, Inc., Cambridge, MA, USA, ⁴Amgen, Inc., South San Francisco, CA, USA

103 Drug Candidate-Induced Changes in the Thyroid Gland: Contrasting Case Studies

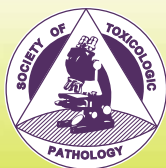
Joan Lane¹, Doriana Froim¹, Daniel Aleksandrowicz¹, Jeffrey Horrigan¹, Katie Zokowski¹, Ken Loveday¹, David Peters¹, Evelyn Polack¹, ¹Biogen Idec, Inc., Cambridge, MA, USA

104 Characterization of a Minipig Model of Gastrointestinal Acute Radiation Syndrome Using Total Body Irradiation and Partial Body Irradiation: A Focus on Intestinal Pathology

Julius Haruna¹, Wieslaw Wierzbicki², Mylene Pouliot¹, Leanne Bossett¹, Alexis Ascah¹, Simon Authier¹, ¹CiToxLAB North America, Laval, QC, Canada, ²Centre Vétérinaire DMV, Lachine, QC, Canada

105 Mechanisms of Acquired Resistance to Toceranib Phosphate (Palladia®) in Canine Mast Cell Tumor

Charles Halsey¹, Daniel Gustafson¹, Barbara Rose¹, Robert Burnett¹, Dawn Duval¹, Anne Avery¹, Donald Backos², Philip Reagan², Douglas Thamm¹, ¹Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO, USA, ²University of Colorado Health Science Center, Aurora, CO, USA



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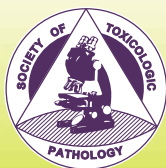
Protective Role of Sildenafil Against Carbon Tetrachloride-Induced Nephrotoxicity by Augmenting the Availability of Nitric Oxide and Antioxidant Enzymes

Category: General Pathology/Toxicologic Pathology

Shubham Goyal¹, Vaneeta Rani², Sawati Sharma¹, Nitin Verma¹

¹*School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences and Technology, Vill-Makhnumajra, Baddi, Distt. Solan, HP, India,* ²*Himalyan Institute of Pharmacy, Kala-Amb, Distt. Sirmour, HP, India*

Introduction: Nephrotoxicity in experimental animals can be induced by CCl₄ (carbon tetrachloride) which is a colorless, volatile and nonflammable liquid of industrial use. Free radicals are generated due to the metabolic transformation of CCl₄ that results in prominent changes in the morphology of kidney, including tubulointerstitial fibrosis and vascular congestion. The present study was designed to investigate the possible mechanism involved in the protective effect of sildenafil (PDE-5 inhibitor) in CCl₄ induced nephrotoxicity. **Material and Methods:** Wistar albino rats of either sex (180-260g), n=6 were employed in the study. Nephrotoxicity was induced by administration of carbon tetrachloride (0.5 ml/kg, s.c.,) for 28 days. Serum creatinine, BUN, urinary microproteins, TBARS, nitrite/nitrate and reduced glutathione estimations were done as hallmarks of renal functioning. **Results:** Administration of CCl₄ induced prominent changes in the morphology of kidney, including tubulointerstitial fibrosis and vascular congestion, increases in serum creatinine, BUN, urinary microproteins, and renal tissue TBARS levels in comparison to normal control. It also decreased reduced glutathione and tissue nitrite/nitrate levels. Sildenafil treatment (0.4 and 0.8 mg/kg) antagonized the effect of CCl₄ induced renal toxicity dose dependently. L-NAME (nitric oxide synthase inhibitor) treatment significantly reversed the effect of sildenafil treatment. **Conclusion:** Therefore, it may be concluded from the above findings that sildenafil has a protective effect in the prevention of renal injury by increasing the availability of nitric oxide and antioxidant enzymes. **Impact statement:** Based upon our evaluation, sildenafil shows a promising result for the treatment of CCl₄ induced nephrotoxicity.



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Effects of Rosiglitazone on β -Cell Function in Metabolic Syndrome Patients with Impaired Glucose Tolerance

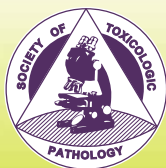
Category: General Pathology/Toxicologic Pathology

Ravinesh Mishra¹, Anees A Siddiqui², Asif Husain², Mohd Rashid¹, Viny Srinivasan³

¹School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences and Technology, Makhnumajra, Baddi, Distt. Solan, HP, India, ²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi, India, ³Department of US Safety Evaluation, L'Oréal, Clark, NJ, USA

Introduction: The objective of this study was to determine the potential effects of rosiglitazone on beta-cell function in metabolic syndrome patients with impaired glucose tolerance and probe into the possible mechanisms. **Experimental Design and Methods:** Twenty subjects were treated with rosiglitazone 25 mg/day for 2 months. At baseline and after treatment, each subject underwent an IVGTT. The acute insulin response (AIRg), the glucose disappearance rates (K) and the ratio of Dinsulin/Dglucose (DI/DG) were calculated according to IVGTT results. Hyperglycemic clamp study was conducted to determine second-phase insulin response, insulin sensitivity index (ISI) and glucose infusion rate (GIR). Euglycemic hyperinsulinemic clamp study was made to measure the glucose disposal rate (GDR). Plasma glucose, free fatty acids (FFAs), serum insulin and proinsulin levels were measured. **Results:** Acute insulin response was unchanged after treatment, whereas the values of coefficients and DI/DG increased. The second-phase insulin response and GIR both demonstrated marked increments. Rosiglitazone therapy also resulted in improvement of ISI value, and the increment of GDR during the euglycemic hyperinsulinemic clamp was also significant. Furthermore, a decrease in fasting proinsulin level was observed, and plasma glucose, FFAs and serum insulin levels all declined. The increase of DI1/DG1 was positively correlated with the improvement of GDR and a positive relationship was observed.

Conclusions: Short-term rosiglitazone therapy improved beta-cell dysfunction, the mechanism of which might involve the attenuation of insulin resistance. **Impact Statement:** Rosiglitazone therapy might arrest or delay the pathophysiologic process from IGT to frank diabetes in subjects with IGT and metabolic syndrome, but longterm prospective studies are needed to verify this possibility.



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Comparative Toxicity and Efficacy of Engineered Anthrax Lethal Toxin Variants with Broad Anti-Tumor Activities

Category: General Pathology/Toxicologic Pathology

Diane Peters^{1,2}, Benjamin Hoover³, Loretta Grey Cloud¹, Shihui Liu³, Alfredo Molinolo¹, Stephen Leppla³, Thomas Bugge¹

¹National Institute of Dental and Craniofacial Research, Bethesda, MD, USA, ²Tufts University Sackler School of Graduate Biomedical Sciences, Boston, MA, USA, ³National Institute of Allergy and Infectious Disease, Bethesda, MD, USA

Introduction: Engineered anthrax toxins have been previously demonstrated to exhibit broad anti-tumor activity in vivo. Here, we perform the first direct comparison of the safety and efficacy of three unique variants requiring activation by either matrix-metalloproteinases (MMPs), urokinase plasminogen activator (uPA) or co-localized MMP/uPA-activities. **Experimental Design:** C57BL/6J mice were challenged with six doses of engineered toxins via intraperitoneal (I.P.) or intravenous (I.V.) dose routes in order to determine maximum tolerated dose (MTD6) and dose-limiting toxicities. Efficacy studies were performed with six I.P. administrations of engineered toxins in C57BL/6J mice bearing B16-BL6 melanoma syngrafts. **Methods:** To evaluate toxicity, necropsies were performed at study termination including complete gross and histopathological analyses. Anti-tumor efficacy was monitored via tumor measurements paired with terminal blood work and tumor immunohistochemistry to elaborate upon the anti-tumor mechanism and relative efficacy of each toxin. **Results:** MMP-, uPA- and dual MMP/uPA-activated anthrax lethal toxins exhibited the same dose-limiting toxicity; dose-dependent GI toxicity. In terms of efficacy, all three toxins significantly reduced primary B16-BL6 tumor burden, ranging from 32%-87% reduction. **Conclusion:** While target organ toxicity and effective doses were similar amongst the toxin variants, the dual MMP/uPA-activated anthrax lethal toxin exhibited the highest I.P. MTD6 and was 1.5-3-fold better tolerated than the single MMP-activated and uPA-activated toxins. **Impact Statement:** An engineered version of anthrax lethal toxin requiring co-localized activation by MMPs and uPA can be administered safely, has potent anti-tumor activity, and is a promising candidate for further development as an anti-cancer agent.



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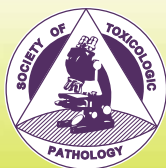
Reproductive Staging in the Göttingen Miniature Pig

Category: General Pathology/Toxicologic Pathology

Rebecca Terry¹, Jan Klapwijk², Franck Chanut²

¹Royal Veterinary College, London, UK, ²GlaxoSmithKline, Ware, UK

Introduction: Minipigs are increasingly popular as a nonrodent species for preclinical testing however limited information is available about the cyclical histologic changes in the female reproductive organs of this species. We aimed to produce a robust, user-friendly guide to reproductive staging in the Göttingen minipig. **Experimental Design:** Sections of ovary, uterus, cervix and vagina from 45 adult nuliparous female Göttingen mini-pigs were blindly evaluated. Each pig was designated as proestrus, estrus-metestrus or diestrus based on ovarian histology and histologic features of the uterus, cervix and vagina were recorded. **Methods and Materials:** Tissues were removed at necropsy, fixed in 10% neutral buffered formalin, trimmed, processed, and embedded in paraffin. Sections were cut, stained with hematoxylin/eosin and evaluated under light microscopy. **Results:** Based on the presence of Graafian follicles, corpora haemorrhagica and the presence, maturation and degeneration of corpora lutea, 18% of animals were found to be in proestrus, 44% in estrus-metestrus and 38% in diestrus. Apoptosis and mitosis within uterine epithelia and stromal inflammatory infiltrates were highly variable within stages as was cervical histology. Histologic changes within vaginal epithelium were subtle and also variable within each stage. **Conclusion:** The minipig vagina and uterus do not have consistent histologic features on which staging can be based (unlike the rat and dog). Therefore ovarian histology is essential for staging the minipig estrous cycle. **Impact Statement:** We provide a histologic guide to reproductive staging in the Göttingen minipig that should facilitate evaluation of the reproductive system in this species in future.



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Histologic Changes Following Acid Reflux Challenge in Porcine Vocal Folds

Category: General Pathology/Toxicologic Pathology

Abigail Durkes¹, Paul Snyder¹, Preeti Sivasankar¹

¹Purdue University, West Lafayette, IN, USA

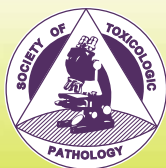
Background: Chronic inflammatory changes are frequently observed in human patients with laryngeal reflux. A standardized reproducible animal model is needed to gain a better understanding of the pathophysiology and histologic changes of laryngopharyngeal reflux [LPR]. We developed an in vivo, uninjured LPR pig model in order to study the histopathology underlying LPR.

Study Design: Prospective animal study.

Methods: Eight pigs were randomly assigned to receive acidified pepsin (pH=4) or saline (control) applied directly to the vocal folds via endoscopic examination. Larynges and associated vocal fold epithelia were collected following three exposures per week for four weeks. The true vocal fold tissue morphology, collagen, and elastin were evaluated histologically by a veterinary pathologist. Virtual slides were created using Aperio ScanScope (Aperio Technologies, Vista, CA). Aperio ImageScope software (v11.2.0.780) quantified the differential staining via pixel counts and the results were standardized over the analysis area. Ultrastructural alterations were examined via transmission electron microscopy (TEM).

Results: After 12 exposures of acidified pepsin over 4 weeks, histologic changes could not be identified in examined specimens. Morphology, collagen, and elastin were not significantly different between control and reflux animals.

Conclusions: Histologic changes were not identified in our pig model following four weeks of reflux exposure. We suspect that either frequency or duration are insufficient to elicit a tissue response. Our results will lead to new understandings of vocal fold changes following LPR exposure and lead to the development of prophylactic treatments to prevent such adverse effects.



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Subchronic Toxicological Study of Two Artemisinin Derivatives in Dogs

Category: General Pathology/Toxicologic Pathology

Ji-ye Yin¹, He-mei Wang¹, Quan-jun Wang¹, Ri-gao Ding¹

¹Beijing Institute of Pharmacology and Toxicology, Beijing, China

Introduction: Artemisinin and its derivatives have consequently been developed as treatments for malaria. They have also been shown to be effective against other parasitic infections and anticancer properties. The objective of this study was to observe the spectrum of toxic changes that result upon the treatment with artemisinin derivatives in long-term administration of low doses. **Experimental Design:** Sixty-four Beagle dogs were randomly assigned to one of four treatment groups: ART-treated (6mg kg⁻¹ per day, orally), ARM-treated (6 mg kg⁻¹ per day, i.m.) and two control group with different treatment route. Termination of treatment was scheduled to occur on weeks 13. **Methods:** Electrocardiograms were recorded using electrocardiogram. Hematology parameters and blood smear were examined. The heart, brain and bone marrow were taken for histopathological examinations. Methods of automated patch clamp technique were used to observe the effects of ART and ARM on the hERG potassium channel in CHO cells. **Results:** Intramuscular administration of 6 mg kg⁻¹ ARM induced a decreased RBC count, and inhibition of erythropoiesis in the bone marrow. We also observed neuropathic changes in the central nervous system, ARM could lead to QT prolongation, which proved the relationship with inhibition of hERG potassium channel. Following treatment with ART, we observed a decreased heart rate, which was most likely due to cardiac conduction system damage, as well as decreased RBC count, and inhibition of erythropoiesis in the bone marrow. **Conclusion:** These findings showed that the prolonged administration of low doses of these derivatives result in diverse toxicity profiles.



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A Maximum Tolerated and Therapeutic Dose of Cholic Acid Rescues the Lethal Effects of Ethanol in the Zebrafish (*Danio rerio*) Fetal Alcohol Model: A Toxicity, Tolerability, and Macroscopic Morphological Study

Category: General Pathology/Toxicologic Pathology

Shemikah Colleton¹, Curtis Colleton², James Marrs⁴, Christian Lawrence³, Courtney Curtis⁴, Kara Maloney³, Mariah Gardner¹, Madeleine Walsh¹, Philip Marx², Lora Becker¹

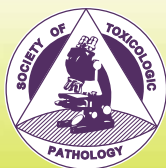
¹University of Evansville, Evansville, IN, USA, ²Bristol-Myers Squibb Company, Mt. Vernon, IN, USA,

³Boston Children's Hospital Aquatic Resources, Boston, MA, USA, ⁴Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA

Introduction: Fetal Alcohol Syndrome (FAS), sublethal embryonic ethanol exposure, characterized by craniofacial, pharyngeal arch and central nervous system abnormalities, can be recapitulated in the developing zebrafish embryo, a valuable model for predictive toxicology and teratogenicity studies.

Objectives: Compare the rescuing potential (tolerability (hatchability), toxicity (% survival) and macroscopic morphological effects) of chronic 10^{-5} and 10^{-11} uM cholic acid (CA) against lethal ethanol (MG) exposures in susceptible zebrafish embryos. **Experimental Design:** Embryos randomized (50-150/25 or 50/group/3 replicates) at ~21 somite-prim 16 into 8 groups: 1) 0% (embryo water), 2) 6% ethanol (MG), 3) CA 10^{-5} uM+DMSO+Methanol, 4) CA 10^{-11} uM+DMSO+Methanol, 5) DMSO (< 0.4%), 6) Methanol (<0.2%), 7) CA 10^{-5} Treatment, and 8) CA 10^{-11} Treatment. Groups 7 and 8 were pre-exposed to MG for 2 hours, then rescued up to 96 hours post fertilization (hpf). **Methods:** Embryos were randomized to wells (250 μ L), incubated at 30°C (refreshed daily), tabulated survival and hatch incidences, recorded macroscopic morphological changes using digital imagery, verified results with appropriate statistical analyses. **Results:** Percent survival (groups): 1) 80-85%, 2) 11-35%, 3) 75%, 4) 42%, 7) 64%, 8) 52%; Percent hatched (groups): 1) 80-85%, 2) 10-36%, 3) 73%, 4) 76%, 7) 57%, 8) 49%; macroscopic morphological findings (primarily pericardial and yolk sac edema) were comparable.

Conclusion: The adverse tolerability, toxicity and macroscopic alterations of lethal MG exposure for up to 96 hpf were rescued comparably with CA 10^{-11} or CA 10^{-5} . **Impact statement:** This model can be used to screen candidates for rescuing the embryonic effects of FAS in humans.



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Chronic Comparative Embryonic Tolerability, Toxicity, and Macroscopic Morphological Study of Sub-Lethal Rectified and Medical Grade Ethanol Exposure in Zebrafish (*Danio rerio*)

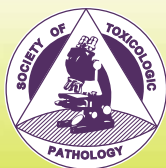
Category: General Pathology/Toxicologic Pathology

Shemikah Colleton¹, Curtis Colleton², James Marrs⁴, Christian Lawrence³, Courtney Curtis⁴, Kara Maloney³, Madeleine Walsh¹, Mariah Gardner¹, Philip Marx², Lora Becker¹

¹University of Evansville, Evansville, IN, USA, ²Bristol-Myers Squibb Company, Mt. Vernon, IN, USA,

³Boston Children's Hospital Aquatic Resources Program, Boston, MA, USA, ⁴Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA

Introduction: The estimated prevalence of Fetal Alcohol Syndrome (FAS) is 0.5-2.0 cases per 1,000 births (USA) with defects of developmental delays, infant mortality and mental retardation. Over a lifetime, 1 child with FAS accrues costs of over \$2 million while the overall costs of FAS is \$0.2 to \$11.7 billion annually. Most FAS preclinical studies are based on exposure to medical grade (MG) ethanol. There are limited preclinical reports of rectified spirits (RS) and numerous clinical reports involving prenatal exposure to RS (tequila, vodka, beer, and wine) but few comparative studies in this model. **Objective:** (1) Compare the tolerability (hatchability), toxicity (% survival), and macroscopic morphological effects of sub-lethal RS and MG exposures in zebrafish embryos. **Experimental Design:** Embryos randomized (100-120/25 or 50/group/3 replicates) at ~21 somite-prim 16 into 3 groups: 1) 0% (embryo water), 2) 2.4% ethanol (MG) and 3) 2% vodka for 96 hours post fertilization (hpf). **Methods:** Embryos were randomized to wells (250 μ L), incubated at 30°C (refreshed daily), survival and hatch incidences recorded, macroscopic morphological changes tabulated; results with appropriate statistical analyses. **Results:** Percent survival (groups): 1) 69%, 2) 59%, 3) 59%; Percent hatched (groups): 1) 68%, 2) 55%, 3) 57%; macroscopic morphological findings (primarily axial deviation) were comparable. **Conclusion:** The tolerability, toxicity, and morphological effects of sub-lethal embryonic exposures to RS and MG were comparable. **Impact statement:** This model can be used to study embryonic exposure to rectified spirits and for screening candidates for rescuing the effects of FAS in humans.



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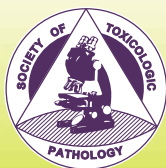
Perfusion Recovery in a Mouse Model of Hind Limb Ischemia Is Enhanced by Mesenchymal Stem Cell-Laden Alginate Implants

Category: New Technologies

Artem Shkumatov¹, Min Kyung Lee¹, Hyun Joon Kong¹

¹University of Illinois, Urbana-Champaign, IL, USA

Introduction: Peripheral artery disease is caused by obstruction of large arteries in the lower limbs and develops as a complication of diabetes. In a mouse model of hind limb ischemia, resection of femoral artery leads to a 90% reduction of blood flow. Human mesenchymal stem cells (MSCs) are used for long term delivery of angiogenic factors into the ischemic region. To shield MSCs from the immune response, MSCs are encapsulated within natural polymer alginate “biopatches”, which can also incorporate different biologically active molecules. **Experimental design:** Four groups (four C57BL/6 mice each) received the following implants after induction of ischemia: microchannelled with IFN γ , microporous (random pores) with IFN γ and MSCs, microchannelled with IFN γ and MSCs, and microchannelled without IFN γ and with MSCs. All animals were sacrificed at the end of week 5. **Methods:** Blood perfusion recovery was evaluated using Laser Doppler perfusion imager (LDPI). Sections of thigh and calf muscles were stained for smooth muscle actin, and the vascular density and area were quantified using NIH Image J software. **Results:** Microchannelled implants with MSCs regardless of IFN γ promoted significantly faster recovery of perfusion and induced an almost two-fold increase in vessel density and total vascular area. Addition of IFN γ was associated with lower incidence of neutrophilic inflammation near implant. Live MSCs were detected in the group without IFN γ at the end of week 5. **Conclusion:** Microchannelled alginate gels with MSCs are a promising technology for angiogenic factor delivery. The role of IFN γ in suppression of neutrophilic response should be further investigated.



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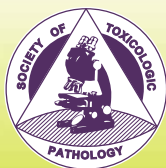
Development of a Tissue Image Analysis Algorithm for Celiac Drug Development

Category: New Technologies

Erik Hagendorn¹, Christa Whitney-Miller², G. David Young¹, Steve Potts¹

¹Flagship Biosciences, Boulder, CO, USA, ²University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

Introduction: Celiac disease, an autoimmune condition related to gluten sensitivity, is gaining pharmaceutical development interest. Recent conversations with the FDA indicate pathology readouts from intestinal biopsy will continue to be a clinical trial endpoint. The existing methodology, the Marsh score, is a qualitative assessment of celiac severity, combining a morphological criteria known as a villous-height to crypt-depth ratio (VC), with an assessment of localized immune response, manually estimating intraepithelial lymphocyte counts (IEL). A stereology and image analysis based whole slide imaging methodology was developed for use in CLIA based clinical trials. **Experimental Design:** 15 jejunal biopsies were manually evaluated by a pathologist to determine Celiac Disease (CD) state using the standard Marsh score, as well as manually measured VC. An automated stereological methodology was used to evaluate surface area on whole slide images. **Methods:** Stereology line probes were used to count one dimensional “hits” on points at the distal ends of the lines which exist over reference tissue area, and “cuts” through the two dimensional range of the line as it passes through the epithelium of the reference tissue to background, or vice versa. **Results:** There was strong concordance between the pathologist scores, and the automated stereology analysis, with the automated approaches able to sufficiently delineate intermediate grades of disease, normally more difficult in visual assessments. **Conclusion:** The quantitative methodology is a valuable addition to CLIA based clinical trials. **Impact statement:** Quantitation provides reproducible and unbiased endpoints that can evaluate both the morphological and immune response in therapeutic clinical studies.



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Wnt Signaling in Prostate Cancer Bone Metastasis

Category: Oncology/Carcinogenesis

Jessica Simmons¹, Wessel Dirksen¹, Thomas Rosol¹

¹The Ohio State University, Columbus, OH, USA

Introduction: The molecular mechanisms by which prostate cancer cells metastasize and grow in bone are not fully understood, however we hypothesized that the Wnt signaling pathways play an important role in the pathogenesis. To investigate the contribution of the Wnt signaling pathways in prostate cancer bone metastases, we over-expressed the Wnt/JNK pathway agonist, Dkk-1, in the mixed osteoblastic and osteolytic Ace-1 prostate cancer cells. Previous work had shown that Dkk-1 expression increased the number and lytic nature of bone metastases in vivo. This study focused on elucidating how enhanced Wnt/JNK signaling could be altering metastasis and the bone microenvironment.

Methods and Experimental Design: Ace-1 cells stably expressing human DKK-1 or empty vector were cultured in vitro. Wnt/JNK signaling was investigated by AP-1 reporter activity, Affymetrix mRNA microarray, and qRT-PCR. Treatment with a non-canonical Wnt agonist and antagonist were performed and the resultant changes in reporter activity, gene expression, proliferation and migration were investigated.

Results: DKK-1 significantly increased non-canonical Wnt/JNK signaling. Subsequent gene expression alterations include a dramatic decrease in mRNA expression of genes important in osteoblast maturation. Treatment with a Wnt/JNK agonist enhanced tumor cell proliferation and migration; this effect was reversed with the antagonist treatment.

Conclusion The present study showed that DKK-1 is a potent activator of non-canonical Wnt/JNK signaling and provides possible mechanisms whereby DKK-1 expression inhibits bone growth and enhance tumorigenesis in prostate cancer metastases.

Impact Statement: This research highlights a potential pathway to target to reduce the morbidity and mortality of prostate cancer bone metastases.



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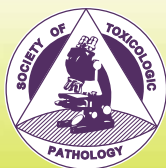
Elucidating the Carcinogenic Mode of Action of Diuron on Rat Urothelium

Category: Oncology/Carcinogenesis

Mitscheli Da Rocha^{1,2}, Lora Arnold², Puttappa Dodmane², Maria Luiza de Oliveira¹, Ana Paula Cardoso¹, Merielen Pontes¹, Karen Pennington², David Muirhead², Fang Qiu², Samuel Cohen², João Lauro de Camargo²

¹São Paulo State University, Botucatu, Brazil, ²University of Nebraska Medical Center, Omaha, NE, USA

Diuron, a substituted urea herbicide, is carcinogenic to the urinary bladder of Wistar rats at high dietary levels, with a higher incidence in males. The proposed carcinogenic mode of action (MOA) includes urothelial cytotoxicity that results in necrosis followed by sustained regenerative cell proliferation and urothelial hyperplasia. To further elucidate the diuron urothelial MOA in male Wistar rats, successive studies aimed to: 1) evaluate the possible influence of urinary solids on the development of urothelial lesions; 2) determine the time course and sequence of bladder cytotoxic and proliferative changes; and 3) evaluate the toxicity of the main diuron metabolites on the urothelium. Rats treated with diuron and NH₄Cl showed decreased urinary pH and reduced amounts of urinary solids. No difference in the incidence of urothelial lesions was found between groups, indicating that cytotoxicity is not due to urinary solids. Rats treated with diuron in a short-term study showed urothelial cell swelling beginning on day 1. Swollen cells at day 7 presented degenerative changes characteristic of cytolysis. At the 8th week, the urothelium showed necrosis, exfoliation and increased incidence of simple hyperplasia. The metabolite DCPU was found in rat urine at concentrations above the *in vitro* IC₅₀ evaluated in the rat MYP3 urothelial cell line. DCPU induced more alterations of gene expression than the other metabolites in MYP3 cells. Taken together, these results indicate that the urothelial carcinogenic MOA of diuron encompasses biotransformation to cytotoxic metabolites, urothelial cell degeneration, necrosis and exfoliation, followed by sustained regenerative cell proliferation, urothelial hyperplasia and eventually tumors.



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ALDH1B1 Is Crucial for Colon Tumorigenesis by Modulating Wnt/ β -catenin, Notch and PI3K/Akt Signaling Pathways

Category: Oncology/Carcinogenesis

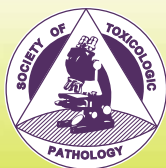
Surendra Singh¹, John Arcaroli², Ying Chen¹, David Orlicky⁴, David Thompson³, Wells Messersmith², Vasilis Vasilou¹

¹Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, ²Division of Medical Oncology, University of Colorado School of Medicine, Aurora, CO, USA,

³Department of Clinical Pharmacy, University of Colorado Anschutz Medical Campus, Aurora, CO, USA,

⁴Department of Pathology, University of Colorado School of Medicine, Aurora, CO, USA

Introduction: Aldehyde dehydrogenase 1B1 (ALDH1B1) is expressed only at the crypt base, along with stem cells in human colon. It is highly expressed in all cancerous cells of human colonic adenocarcinomas. This pattern of expression corresponds closely to that observed for Wnt/ β -catenin signaling activity in normal and cancerous colon. **Methods:** 3-kb promoter region of human ALDH1B1 gene was analyzed for candidate T-cell factor/lymphoid enhancing factor (TCF/LEF) binding elements and dual luciferase reporter assay was used to analyze ALDH1B1 promoter activity in colon cancer cells. ALDH1B1 was knocked down using shRNA in SW480 cells and used these cells for 3-dimensional spheroid growth and nude mice xenograft studies. We examined Wnt reporter activity and protein/mRNA expression for Wnt, Notch and PI3K/Akt signaling pathways. Flow cytometric analysis was conducted using Aldefluor assay on ALDH1B1 shRNA-transfected SW480 cells. **Results:** The shRNA-mediated knockdown of ALDH1B1 reduced the number and size of spheroids formed by colon cancer cells. ALDH1B1 knockdown depletes the highly carcinogenic ALDHbright cells and significantly decreased xenograft tumor formation in athymic mice. Wnt/ β -catenin, Notch and PI3K/Akt-signaling pathways were down-regulated in ALDH1B1-depleted colon cancer cells. **Conclusion:** In summary, our data demonstrate that ALDH1B1 plays a functional role in colon cancer tumorigenesis by modulating the Wnt/ β -catenin, Notch and PI3K/Akt signaling pathways. **Impact statement:** For the first time we have found that ALDH1B1 is not only a potential biomarker for colon cancer but is crucial for colon carcinogenesis by which, these cancer cells could be selectively targeted, leading to more effective treatments for this condition.



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Cigarette Smoke Condensate Induces Early Epithelial-Mesenchymal Transition (EMT) in Cultured Human Ectocervical Cells

Category: Oncology/Carcinogenesis

Xiaohua Gao¹, Linda Yu¹, Lysandra Castro¹, Deloris Sutton¹, Connie Cummings¹, Daniel Morgan¹, Grace Kissling², Darlene Dixon¹

¹NIEHS, NTP, Research Triangle Park, NC, USA, ²NIEHS, Research Triangle Park, NC, USA

Introduction: Numerous studies have demonstrated that cigarette smoking is an important risk factor for cervical cancer, albeit the underlying mechanism(s) remains unclear. Also, tobacco components have been found in cervical mucus of women smokers. The aim of our study was to examine potential direct effects of cigarette smoke condensate (CSC) on a human ectocervical cell line (Ect1/E6E7).

Experimental Design: Ect1/E6E7 cells were exposed to CSC for 24h, 72h and 168h. **Methods:** Cell proliferation was measured using MTS assays. Histomorphologic and ultrastructural changes were determined by light microscopy and TEM, respectively. E-cadherin expression, a sensitive marker of EMT, critical for the progression of cervical and other cancers, was evaluated by confocal immunofluorescence. **Results:** CSC did not significantly induce cell proliferation; however, in H&E sections we observed that cells exposed to CSC (10 µg/mL) lost their “cobblestone” morphology and became “spindle-like” by 72h. Confocal immunofluorescence studies showed a significant reduction in E-cadherin-positive fluorescence signals in CSC-treated cells, compared to controls at 24h (100±8.6% vs. 59.5±3.4%, respectively, p<0.01), which remained reduced at 72h and 168h. Interestingly by TEM, CSC-treated cells were significantly enlarged nearly 1.5 times that of controls (329.48±278.1 vs. 237.2±182.5µm²/cell, respectively, p≤0.05). Additionally, these cells had decreased surface filapodia, cytoplasmic swelling, single membrane vacuoles, and variable-sized mitochondria, which are ultrastructural changes compatible with EMT. **Conclusion:** Our data suggest that CSC induces EMT in human cervical epithelial cells. **Impact Statement:** EMT may be a novel molecular mechanism of early cellular transformation and implicates cigarette smoking as a risk factor for cervical cancer.



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Identification of Differentially Expressed Genes Defining Heterogeneity of Cancer Cells by RNA-Seq using Next Generation Sequencing

Category: Oncology/Carcinogenesis

Yongbaek Kim^{1,2}, Myung-Chul Kim¹, Na-Yon Kim¹, Hang-A Kim¹, Cui-Feng Ji¹

¹Laboratory of Clinical Pathology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea, ²Research Institution of Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

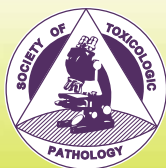
Introduction/Objectives: Intratumoral heterogeneity is common in all tumor types and is a major barrier against effective cancer therapy. Especially, human malignant mesothelioma that is an environmental contaminant-induced disease is known to be extremely heterogeneous with regards to morphology as well as molecular phenotypes, resulting in poor response to cancer therapy. This study was performed to investigate the global gene expression profile of distinct subpopulations of MS1, a human malignant mesothelioma cell line.

Materials and Methods: The MS1 cells were cultured in complete media and subjected to the side population (SP) assay composed of Hoechst 33342 dye staining and subsequent flow cytometry. Total RNAs were isolated from the sorted subpopulation cells, SP and non-SP fractions, followed by mRNA purification and fragmentation into smaller length. Following construction of RNA-seq library by reverse transcription and PCR amplification, the library was sequenced using Illumina HiSeq2000. After processing of the raw data, high quality reads were subjected to the mapping of the human genome, followed by analysis of differentially expressed genes (DEGs).

Results: A total of 1130 genes including 795 upregulated and 335 down regulated were identified based on the criteria of two-fold difference and a p-value <0.05. In ontology analysis, the large portions of the DEGs were belonged to the groups of cytoskeleton and intracellular signaling cascade.

Conclusion: The RNA-seq using next generation sequencing technology revealed DEGs between SP and NSP fractions.

Impact statement: This study will provide background information to promote the development of an innovative strategy to eliminate more aggressive cancer cell subpopulations.



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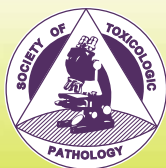
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An Allelic Variant of the Mechanistic Target of Rapamycin (mTOR) Leads to Altered DNA Damage Repair

Category: Oncology/Carcinogenesis

Joy Gary^{1,2}, Shuling Zhang¹, Ke Zhang¹, Wendy Dubois¹, Aleksandra Michalowski¹, Beverly Mock¹
¹CCR, NCI, NIH, Bethesda, MD, USA, ²Michigan State University, East Lansing, MI, USA

Introduction: Our lab discovered an allelic variant of Mtor (C1977T; Mtor^{tm1.1Lgm}) in BALB/cAnPt mice, which predisposes these mice to the development of pristane-induced plasmacytomas. This allelic variant results in a single amino acid substitution (R628C). **Materials and Methods:** A knock-in (KI) mouse was developed by homologous recombination that carries the BALB/c allele (1977T) of Mtor on a B6;129 background. **Experimental Design:** mRNA expression in the bone marrow was compared between pristane-treated WT and KI mice. Ingenuity Pathway Analysis was used to determine top enriched networks in differentially expressed genes, which included DNA damage response (DDR). DDR was evaluated in 628C KI mice and in embryonic fibroblasts. **Results:** Network enrichment analysis of microarray results revealed that DNA replication and repair was one of the top enriched networks. To explore the DDR, 628C KI and WT mice were irradiated; KI male mice had decreased survival. WT and KI mouse embryonic fibroblasts (MEFs) were immortalized with SV40 Large T antigen and irradiated. The surviving fraction was significantly decreased in KI MEFs. MEFs from 628C KI mice had increased phospho-H2AX compared to WT MEFs. **Conclusion:** The allelic variant of mTOR affects the DDR. **Impact Statement:** mTOR has previously only been tangentially associated with DDR.



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Possible Mechanisms Underlying Exacerbation of Osmotic Nephrosis Caused by Pre-Existing Kidney Injury

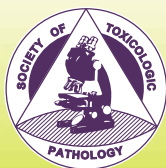
Category: Systemic/Organ-Specific Toxicologic Pathology

Kohei Matsushita¹, Shinji Takasu¹, Yuji Ishii¹, Ken Kuroda¹, Aki Kijima¹, Keisuke Kitaura², Makoto Sato², Satoshi Matsumoto², Kumiko Ogawa¹, Takashi Umemura¹

¹Division of Pathology, National Institute of Health Sciences, Tokyo, Japan, ²Safety Research Center, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan

Introduction: Osmotic nephrosis is clinically induced by plasma expander including dextran, and histologically characterized by swollen and vacuolated proximal tubules, i.e. clear tubules (CLs). This study investigated the mechanisms of exacerbation of osmotic nephrosis caused by kidney injury.

Experimental design and methods: Experiment I; Male SD rats (6-weeks-old) were given gentamicin for 6 days (125 mg/kg, ip), followed by dextran treatment for 14 days (4000 mg/kg, iv). Two additional groups were given gentamicin or dextran alone. The kidneys were examined by light and electron microscopy. Experiment II; Female F344 rats (10-weeks-old) were subject to ischemia/reperfusion in the left kidney and sacrificed after 3 or 7 days. Dextran (4000 mg/kg, iv) or saline were given 1 day before sacrifice, followed by histopathology. **Results:** Experiment I; Gentamicin induced regenerative tubules (RTs), and dextran induced vacuolated proximal tubules and CLs. While the incidence of RTs decreased, that of CLs increased with co-treatment. Ultrastructurally, vacuolation showed secondary lysosomes containing dextran, while CLs demonstrated over-accumulation of dextran in RTs. Experiment II; RTs were classified morphologically into stages I (early phase) and II (terminal phase). In saline-treated rats, stage I appeared predominantly at day 3, and stage II at day 7. Dextran induced many CLs at day 3 and stage II vacuolation at day 7. **Conclusion and impact statement:** CLs may develop selectively in RTs at early stage, which may explain the mechanism of exacerbation of osmotic nephrosis caused by kidney injury. Gene expression analysis to investigate intracellular environment of stage I and II are ongoing.



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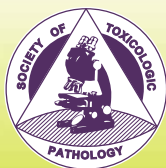
Role of Cysteine-Rich Secretory Protein LCCL Domain Containing 2 [CRISPLD2] in Bile Duct Epithelial Branching Morphogenesis during Hepatic Fibrosis and Cholangiocarcinoma Progression

Category: Systemic/Organ-Specific Toxicologic Pathology

Chandrassegar Saravanan¹, Cheryl Spence¹, James Trevaskis¹, Xiaosong Wang¹, Jean-Rene Galarneau¹, William Chutkow¹, Keith Mansfield¹

¹Novartis Institutes for Biomedical Research, Cambridge, MA, USA

Introduction: Studies in the past have shown that the degree of hepatic fibrosis positively correlates to the extent of bile duct hyperplasia in nonalcoholic steatohepatitis (NASH). However, the mechanisms of cross-talk between the bile ductules and the hepatic stellate cells during NASH progression were not established. **Experimental Design:** Liver samples from leptin-deficient mice fed with high fat diet or low fat diet and human patients with cholangiocarcinoma were used. **Methods:** The immunohistochemistry and in situ hybridization were performed on automated tissue staining platforms. Multispectral imaging system, ImageScope, and Definiens Developer softwares were used for image analyses. Affymetrix GeneChip (Mouse MG_430 2.0), compare, array mining tool, and IPA were used for microarray analysis. **Results:** The bile ductules were spatially associated to pericellular fibrosis in mouse and human NASH liver tissues. The gene encoding CRISPLD2, a secretory protein reported to be critical for epithelial morphogenesis during lung and kidney development, was among the top differentially up regulated genes in the mouse model of NASH. CRISPLD2 expression was localized in the myofibroblasts that accompany the bile ductules and pericellular fibrosis. Furthermore, cancer-associated myofibroblasts express CRISPLD2 in human cholangiocarcinoma tissues. **Conclusions:** CRISPLD2 secreted by the myofibroblasts may play a role in branching morphogenesis of bile ductules during hepatic fibrosis in NASH and in cholangiocarcinoma progression. **Impact statement:** Further characterization of the role of CRISPLD2 may help us to understand the epithelial-mesenchymal interactions between bile ductules and hepatic stellate cells during progression of NASH and cholangiocarcinoma.



Macrophage-Derived Galectin-3 Is the Key Regulator of Acute Hepatic Fibrogenesis in Rats

Category: Systemic/Organ-Specific Toxicologic Pathology

Hossain M. Golbar¹, Takeshi Izawa¹, Bondoc Alexandra¹, Kavindra K. Wijesundera¹, Anusha H. Tennakoon¹, Chisa Katou-Ichikawa¹, Miyuu Tanaka¹, Mitsuru Kuwamura¹, Jyoji Yamate¹

¹Osaka Prefecture University, Izumisano City, Osaka, Japan

Introduction: Galectin (gal)-3 is a β -galactoside-binding animal lectin involved in cell to cell and cell to matrix interactions. Galectin (gal)-3 expressions was assessed in Kupffer cell-depleted livers after acute hepatic damage by immunohistochemistry, immunofluorescence and molecular analysis. **Methods and Materials:** Kupffer cells were depleted by liposomal chlodronate (CLD; 10 mL/kg BW, i.v.) one day before induction of hepatocyte injury by thioacetamide (TAA; 300 mg/kg, i.p.) or cholangiocyte injury by α -naphthylisothiocyanate (ANIT; 75 mg/kg, i.p.) in 6-week-old male F344/DuCrIj rats; the control rats received empty liposome followed by TAA or ANIT. Liver samples were processed for histopathology, immunohistochemistry (Iba-1, gal-3, vimentin, desmin, α -SMA), real-time RT-PCR (Iba-1, gal-3, IL-10, α -SMA, Col1 α 1), and immunofluorescence staining (gal-3 with CD68, CD163, MHC class II and CD204; α -SMA with vimentin and desmin). **Experimental Design:** Forty-eight rats were included in CLD-induced macrophage depletion group and 48 rats in empty liposome treated control group. Forty rats of the macrophage depletion group were injected with either TAA (20 rats) or ANIT (20 rats) and euthanized under deep isoflurane anesthesia on days 1, 2, 3, 5 and 7 (n = 4 at each examination point). The remaining 8 rats in macrophage depleted group received vehicle of TAA (4 rats) or ANIT (4 rats) and euthanized immediately (day 0). Similarly, the rats in the control group received either TAA or ANIT and sacrificed accordingly. **Results:** Depletion of Iba-1⁺ macrophages resulted in depletion of gal-3⁺ cells on day 0. After injury, gal-3⁺ cells significantly increased (P<0.05) with the peak on day 2 in control rats while they were fewer in macrophage-depleted rats. Vimentin⁺, desmin⁺ and α -SMA⁺ myofibroblastic cells appeared later in macrophage-depleted rats compared to controls. Real-time RT-PCR data were supportive of immunohistochemical findings. Double labeling exhibited various immunophenotypes of gal-3⁺ cells and myofibroblasts. **Conclusion:** Macrophages are the primary source of gal-3 and its absence attenuates fibrosis. **Impact:** Macrophages or Kupffer cells may represent potential target for anti-fibrotic therapy.



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Improved LV Function in Levosimendan-Treated Rats with Reversed Volume Overload Heart Failure Correlates with Normalized Alpha-to-Beta Myosin Heavy Chain Expression

Category: Systemic/Organ-Specific Toxicologic Pathology

Kristin Wilson^{1,2}, Mary Cismowski^{1,2}, Pamela Lucchesi^{1,2}

¹The Ohio State University, Columbus, OH, USA, ²Nationwide Children's Hospital, Columbus, OH, USA

Introduction. In aortocaval fistula (ACF)-induced volume overload (VO) heart failure (HF), surgical intervention during pre-HF (4 weeks post-ACF) and established HF (8 weeks post-ACF) results in delayed to absent left ventricular (LV) functional recovery, respectively. Myosin heavy chain (MHC) isoform is intimately involved in cross-bridge cycling kinetics, and α -MHC switches to β -MHC in HF. This study investigated if pre- or post-surgical treatment with levosimendan (Levo; myofilament Ca^{2+} sensitizer) could alter LV functional recovery and alter α -to- β -MHC expression. **Experimental Design/Methods:** At Week 0, ACF or Sham surgery was performed in male Sprague-Dawley rats (200–240 g). In one subset, the ACF was reversed (REV; closed) at Week 4 and given Levo (1 mg/kg in drinking water) or Vehicle (water) from Weeks 4–8. Additional subsets were reversed at Week 8 and given Levo from Weeks 4–8, Levo from Weeks 8–19 or Vehicle. **Results:** In ACF, there was progressive LV dysfunction that correlates with decreased α -to- β -MHC. Levo given post-reversal at Weeks 4 and 8 improved LV function at Weeks 8 and 19, respectively, which correlated with normalized α -to- β -MHC, while Levo given pre-REV@8 only transiently improved LV function. In the pre-REV@8-treated rats, LV function was impaired at Week 19, which correlated with decreased α -to- β -MHC at Weeks 8 and 19. **Conclusions:** These results demonstrate that Levo can rescue, but not prevent, post-reversal dysfunction. Normalization of α -to- β -MHC may explain the improved LV function, while decreased α -to- β -MHC may explain the dysfunction in Levo pre-treated rats. **Impact:** Therapeutic strategies that normalize α -to- β -MHC may improve LV function in VO HF.



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The Combined Use of Structural and Functional Cardiac Biomarkers Enhances the Ability to Understand the Pathogenesis of Cardiotoxicity

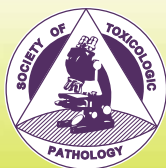
Category: Biomarkers

William Reagan¹, Vincent Bernardo¹, Raju Mantena³, Bernie Buetow², Jianying Wang², Allison Vitsky², Hugh Barton⁴, Jon Heyen², Karen Leach⁵, Dingzhou Li¹, Carrie Northcott¹, David Potter¹, Deb Burt¹, Rick Goldstein¹, Wendy Hu², Nick Edmunds¹

¹Pfizer Drug Safety Research and Development, Groton, CT, USA, ²Pfizer Drug Safety Research and Development, LaJolla, CA, USA, ³Pfizer Drug Safety Research and Development, Cambridge, MA, USA, ⁴Pfizer Pharmacokinetics Dynamics and Metabolism, Groton, CT, USA, ⁵Pfizer Compound Safety Prediction Group, Groton, CT, USA

Introduction: Structural and functional cardiac biomarkers were used to characterize cardiac toxicity associated with compound X. **Experimental Design:** Wistar rats were dosed daily with compound X (n=10/group), euthanized on Days 17 and 31, and compared to controls. Cardiac troponin I (cTnI), fatty acid binding protein (FABP), myosin light chain 3 (MYL3), skeletal muscle troponin I (sTnI), NT-proANP, miRNA, and functional cardiac endpoints were assessed in-life. Routine clinical pathology, heart weights, light and electron microscopic evaluation (select samples) of the hearts were conducted at necropsy.

Methods: MSD assay was used to measure cTnI, FABP, and MYL3, sTnI. ELISA (Alpco) was used for NTproANP. MicroRNAs miR1, miR133a, miR208a, mi499 were measured by Taqman. Functional cardiac markers, including blood pressure (BP) and heart rate (HR), were assessed by telemetry. **Results:** Pharmacologically mediated decreases in cholesterol and triglycerides were present on Days 17 and 31. Increases in HR and BP occurred on Days 1, 15 and 29. Increases in cTnI were present on Day 2 and NT proANP was increased on Days 16 and 30 and corresponded to heart weight increases on Day 31. There were no test article-related changes in miRNA, FABP, MYL3 and sTnI. The hearts showed no cardiac necrosis or cardiac hypertrophy but myocyte vacuolation on Day 31 corresponded with mitochondrial dropout on electron microscopy. **Conclusion:** Compound X induced functional and structural changes to the heart which could be monitored with fluid-based biomarkers and telemetry. **Impact Statement:** Combining the assessment of structural and functional cardiac biomarkers can be used to better understand the pathogenesis of cardiotoxicity.



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Determination of Atrial Natriuretic Peptide in Sprague-Dawley Rat and C57BL/6 Mouse Serum: Comparison Between Two Immunoassays

Category: Biomarkers

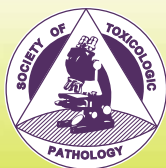
Ahmad Farhad¹, Mehrdad Ameri¹, Jim Turk¹

¹Comparative Biology and Safety Sciences, Amgen, Inc., Thousand Oaks, CA, USA

Introduction: Atrial natriuretic peptide prohormone (proANP) is synthesized by cardiomyocytes of normal atrium and hypertrophic ventricle. ProANP is cleaved in circulation into active peptide and inactive N-terminal peptide (NT-proANP). NT-proANP has a long half-life and is less sensitive to the pulsatile secretions of ANP, making it a useful biomarker to evaluate cardiac health. The objective of this study was to investigate two commercial immunoassays for NT-proANP measurement in rat and mouse serum.

Experimental Design: Serum samples from 27 male and 70 female Sprague-Dawley rats (10-14 weeks old) and 14 male and 22 female C57BL/6 mice (6-30 weeks old) were included in this study. **Methods:** A rat-specific ELISA from Meso Scale Discovery (MSD) and a human-specific ELISA from ALPCO

Diagnostics were investigated for NT-proANP measurement. **Results:** The coefficient of variation of the MSD and ALPCO assays were <10% and <16%, respectively. Spike-recovery using each kit's standard in pooled rat serum was $110 \pm 10\%$ for the MSD assay and $89 \pm 9\%$ for the ALPCO assay. NT-proANP was stable up to three freeze-thaw cycles and no interference due to hemolysis was observed for either assay. NT-proANP mean values determined by the MSD assay were significantly higher ($p < 0.05$) than the values determined by the ALPCO assay. **Conclusion:** Both assays meet the feasibility acceptance criteria for determination of NT-proANP in rat and mouse sera. **Impact statement:** This study helps to establish an understanding of the performance of two immunoassays for measurement of NT-proANP as a cardiac biomarker in preclinical toxicology investigations.



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Altered Ubiquitin Expression in the Airways of Diacetyl-Exposed Mice

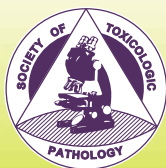
Category: Biomarkers

Ann Hubbs¹, Kara Fluharty¹, Rebekah Edwards¹, John Grantham^{1,2}, Linda Sargent¹, Steven Reynolds¹, Robert Mercer¹, Michael Kashon¹, Lori Battelli¹, Mark Jackson¹, Amy Cumpston¹, Travis Goldsmith¹, David Frazer¹, Tiffani Munro¹, Winnie Moyers¹, Kimberly McKinstry¹, Sherri Friend¹, Krishnan Sriram¹
¹National Institute for Occupational Safety and Health, Morgantown, WV, USA, ²West Virginia University, Morgantown, WV, USA

Introduction: Flavorings-related lung disease is associated with inhaling vapors of diacetyl (2,3-butanedione), an α -dicarbonyl flavoring which reacts with proteins. Cells process misfolded proteins by ubiquitination and proteosomal degradation. However, ubiquitination at lysine 63 (K63-ubiquitination) can activate signaling cascades instead of degrading proteins. We examined the hypothesis that diacetyl inhalation increases total ubiquitin and K63-ubiquitin aggregates in airway epithelium. We also examined the modifying effect of dicarbonyl/L-xylulose reductase (DCXR), which metabolizes diacetyl.

Experimental design: Wildtype and DCXR knockout mice inhaled target concentrations of 0, 100, 200, or 300 ppm diacetyl for 6 hours. **Methods:** At 1 day post-exposure, we counted airway epithelial cells with aggregated immunoreactive total ubiquitin or K63-ubiquitin. In a follow-up experiment, olfactory bulb (OB) *Tnfa* and olfactory marker protein (*Omp*) mRNA and lung *Tnfa* and *Scgb1a1* mRNA were measured 1 day after inhaling 200 ppm diacetyl. **Results:** In terminal bronchioles, cells with aggregated K63-linked ubiquitin increased in wildtype and knockout mice inhaling 200 ppm. In larger bronchioles, all tested diacetyl concentrations increased cells with aggregated total ubiquitin and K63-ubiquitin irrespective of genotype. At 200 ppm, diacetyl increased *Tnfa* in OB of wildtype mice and this was enhanced in knockout mice. Irrespective of genotype, *Omp* decreased in OB, suggesting olfactory neuron loss. In knockout but not wildtype mice, 200 ppm decreased lung *Scgb1a1*, suggesting club cell loss.

Conclusion: Aggregated ubiquitin and K63-ubiquitin suggest biological responses to protein damage. DCXR may modify diacetyl toxicity. **Impact:** K63-ubiquitination in airway epithelium may be a mechanistically-based biomarker of protein damage in airway epithelium.



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Biomarkers of Hypercoagulability in a Rat Sepsis-Induced Model of Non-Overt Disseminated Intravascular Coagulation (DIC)

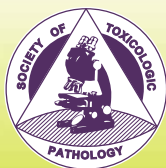
Category: Biomarkers

MB Brooks¹, JR Turk², CE Fishman³, DW Wilson⁴, AE Schultze⁵, JB Pierson⁶, members of the Cardiac Biomarkers Working Group of the HESI Cardiac Safety Technical Committee

¹Cornell University, Ithaca, NY, USA, ²Amgen, Inc., Thousand Oaks, CA, USA, ³GlaxoSmithKline, King of Prussia, PA, USA, ⁴University of California, Davis, Davis, CA, USA, ⁵Eli Lilly and Company, Indianapolis, IN, USA, ⁶HESI, Washington, DC, USA

Introduction: Drug-induced thromboembolic events are serious safety concerns, yet current preclinical test strategies lack predictive biomarkers of drug-mediated prothrombotic states. We modified a model of sepsis-induced DIC in rats to evaluate candidate biomarkers of early stage, procoagulant imbalance.

Experimental Design: Male Wistar rats (n=25 per group) were treated with endotoxin [lipopolysaccharide (LPS), 15 mg/kg, IP] or vehicle control. At 1, 4, 8, 24, and 48 hours 5 rats per group were anesthetized for terminal sampling and necropsy. **Methods:** At each time point we evaluated: CBC, coagulation assays (APTT, PT, fibrinogen), circulating markers of endothelial injury (sE selectin, sICAM-1), flow cytometric parameters of platelet activation (P selectin, bound fibrinogen), and reviewed histologic sections of brain, heart, kidney, liver, spleen, mesentery, and lungs. **Results:** All treated rats survived to 48 hours without significant prolongation of clotting times or signs of hemorrhage that characterize the consumptive coagulopathy of overt DIC. Within 4 hours they displayed clinicopathologic features of acute inflammation, multi-organ neutrophil sequestration, and thrombocytopenia without activation of circulating platelets. sICAM-1 levels rose 4 to 6 fold and were significantly higher ($p < 0.05$) than controls from 4 through 48 hours. **Conclusion:** Endotoxin administration induced an inflammatory stimulus and compensated hypercoagulability that did not overwhelm the mechanisms opposing microvascular fibrin deposition. Soluble ICAM-1 levels rose early and progressively increased in concert with histologic evidence of endothelial activation. **Impact Statement:** Plasma sICAM-1 appears to be a useful early biomarker of endothelial activation/injury in a model of prethrombotic, non-overt DIC.



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Application of Predictive Biomarkers for Renal Toxicity in Drug Development

Category: Biomarkers

Kaoru Toyosawa¹, Takayuki Iwaisako¹, Akiko Kusayanagi¹, Izumi Matsumoto¹, Mami Kouchi¹, Tomoaki Tochitani¹, Toru Yamada¹, Hitoshi Funabashi¹

¹*Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan*

Introduction: Sensitive methods for prediction of renal toxicity in preclinical studies are extremely important for patient safety in clinical practice for medicinal drug development. The present study was conducted to identify predictive biomarkers for our test article that induces histological changes in the kidney, such as regenerative changes in the distal tubule and Henle's loop in rats and monkeys.

Materials & Methods: The dose level was set at 300 mg/kg/day based on the results of the previous 2-week study. Male rats were orally dosed with the test article for 1, 3, 7 or 14 days. Parameters evaluated were urinalysis including new urinary biomarkers, hematology, blood chemistry, necropsy, organ weight and histopathology. **Results:** The test article-related changes were consistent of increases in urine albumin, LDH, Kim-1 and cystatin-C, and increases in plasma BUN, creatinine and cystatin-C. At necropsy, the kidneys were enlarged and white and had increased weights. Regenerative tubules were observed on Days 7 and 14. Among these changes, increases in urine albumin, LDH and Kim-1 were noted on Day 3 when regenerative tubules were not detected at light microscopic examination.

Conclusion: Based on these results, urine albumin, LDH and Kim-1 were considered to be appropriate as predictive biomarkers for renal toxicity induced by our test article. These urine biomarkers were also measured in further 4-week studies and good correlation of these biomarkers with renal toxicity was observed.



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Investigation of Urine Biomarker Performance in Rat Glomerular Injury Models

Category: Biomarkers

Bruce Homer¹, Steven Bailey², Eva Nagiec¹, Deborah Burt³, Mira Ko⁴, Karen Percival², Rounak Nassirpour², Radi Zaher², James Warneke², Thomas Brown³

¹Pfizer, Inc., Cambridge, MA, USA, ²Pfizer, Inc., Andover, MA, USA, ³Pfizer, Inc., Groton, CA, USA,

⁴Pfizer, Inc., La Jolla, CA, USA

Introduction: Rat urine glomerular biomarkers, including microalbumin, total protein, beta 2-microglobulin, Cystatin C, NGAL, C5b-9 and MCP-1 were assessed in two models of glomerular injury: puromycin (PAN) and sheep anti-rat Fx1A induced passive Heymann nephritis (PHN). **Experimental Design and Methods:** PAN and PHN study duration were 11 and 16 days, respectively, with overnight urine collection and necropsy on days 2, 3, 6, and 11 (PAN) or on days 3, 6, 9 and 16 (PHN). Statistical analysis was performed by two-way analysis of variance (ANOVA), followed by Tukey's pair-wise testing for each biomarker collection day. **Results:** Earliest PAN study morphologic changes, observed by electron microscopy (EM) on Day 2, were dense proteinaceous inclusions in podocytes. Associated increases in podocyte PAS positive cytoplasmic granules were observed on Day 3. Other light microscopic (LM) changes were not observed until Day 6. Earliest significant elevations in urine biomarkers were microalbumin (Day 2), Cystatin C and beta 2-microglobulin (Day 3) and total protein (Day 6). Earliest PHN study morphologic changes, observed by EM on Day 3, were subepithelial immune complex deposits. Associated glomerular basement membrane deposition of C5b-9 (IHC) was observed on Day 6. Other LM changes were not observed until Day 9. Earliest significant elevations in urine biomarkers were NGAL and C5b-9 (Day 3), and microalbumin, total protein and beta 2-microglobulin (Day 9). **Conclusion:** Sets of prodromal and correlative rat urine biomarkers were identified. **Impact Statement:** These biomarkers may enable the ability to monitor progression of glomerular injury in nonclinical and clinical studies.



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Unexpected Pituitary Pathology after Chronic Administration of MEDI412, a High Potency Anti-IgE Antibody

Category: General Pathology/Toxicologic Pathology

Mary McFarlane², William Iverson¹, Juerg Bluemel¹

¹MedImmune, LLC, Gaithersburg, MD, USA, ²MedImmune, LLC, Cambridge, UK

Introduction: Elevated serum IgE levels play an important role in allergic asthma and atopic dermatitis. MEDI4212 binds to cynomolgus monkey, but not rodent, IgE with comparable affinity to human IgE.

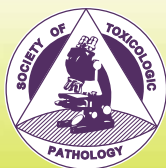
Experimental Design: Three males and three females were treated with weekly SC doses for 26 weeks at 0, 50, or 150 mg/kg. An additional two animals of each gender from each group were untreated for 13 weeks.

Methods: A full set of tissues from all animals were fixed in 10% nbf and processed to H&E slides. Additional sections of pituitary gland were also stained using IHC techniques for MEDI4212, FSH, LH, PrL and Ki67.

Results: Hypertrophy of the pituitary gland was seen in all females at 150 mg/kg (high dose) and in 2/3 animals at 50 mg/kg (low dose). This finding was still present at the end of a 13-week recovery period in high dose group only, but no down-stream functional effects in endocrine organs were observed. Investigative studies confirmed the absence of hyperplasia and demonstrated that MEDI4212 did not bind to circulating female pituitary hormones or pituitary cells.

Conclusion: Although no clear mechanism of action could be identified in these follow-on studies, a NOAEL of 50 mg/kg once weekly was proposed based on (1) lack of functional consequences, (2) low-grade severity of the lesion and (3) slight trend towards recovery.

Impact statement: This study shows that off-target effects can occur in the endocrine system with monoclonal antibodies but these may have no functional or physiologic consequences for the animal.



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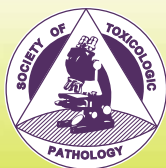
Toxicological Findings in Beagle Dogs after 13 Weeks Oral Gavage of a DPP-4 Inhibitor

Category: General Pathology/Toxicologic Pathology

Jingjun Sun¹, Henglei Lu¹, Jianhong Pan¹, Junwen Qiao¹, Rongrong Tan¹, Hua Sheng¹, Jin Ren¹

¹Center for Drug Safety Evaluation and Research, Shanghai Institute of Materia Medica, Shanghai, China

Introduction: AA-4 is a new DPP-4 inhibitor for treatment of Diabetes type II. This study is intended to provide a preliminary assessment of the toxicity of the AA-4 after 13 weeks repeat dose in the Beagle dogs. In our study, the AA-4 was found targeted mainly on eyes, liver and fur in dogs. **Experimental Design:** Thirty-two Beagle dogs were selected for consecutive oral gavage toxicity study with AA4 for 13 weeks and some animals recovered for 4 weeks. Animals were euthanized for necropsy two days and four weeks after receiving the final dose in main study and recovery phase, respectively. **Methods:** All dogs were euthanized with pentobarbital sodium followed by exsanguination from the femoral arteries and veins. After fixation, tissues were trimmed, dehydrated, embedded, sectioned into slides and stained with hematoxylin/eosin. **Results:** Macroscopically, all the animals at the dose of 1000 mg/kg/day showed fur color lightened and eyes color darkened in the main study. In the recovery study, both animals in the 300 mg/kg/day and 1000 mg/kg/day showed fur color lightened and 1 animal in 1000 mg/kg showed eyes color darkened. Four in six animals were found microscopically to have slightly hypertrophied tapetal cells at the dose of 1000 mg/kg/day in the main study. **Conclusion:** At the dose of 1000 mg/kg/day, the tapetal cells hypertrophy slightly and no changes were found after the recovery period. **Impact statement:** The changes in tapetum lucidum may have no relevance with human because human eyes have no tapetum lucidum.



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Dysregulation of Iron Homeostasis during Progression of Thioacetamide-Induced Liver Cirrhosis in Rats

Category: General Pathology/Toxicologic Pathology

Takeshi Izawa¹, Hiroshi Murakami¹, Rena Miyagi¹, Kavindra Wijesundera¹, Anusha Tennakoon¹, Golbar Hossain¹, Mitsuru Kuwamura¹, Jyoji Yamate¹

¹Osaka Prefecture University, Osaka, Japan

Introduction: Hepatic iron overload is a risk factor for cirrhosis and hepatocellular carcinoma in human chronic liver diseases. However, the pathogenesis is still largely unknown. **Experimental design:** Six-week-old male F344/DuCrIj rats were injected intraperitoneally with thioacetamide (100 mg/kg, twice a week) and saline (for control). **Methods and Materials:** Liver and serum were collected at weeks 5, 7, 12, 15 and 25 (n=4 in each group). Liver sections were stained with HE and Sirius red for histopathologic examination. Liver and serum iron were analyzed biochemically. Hepatic expression of hepcidin (a central regulator of systemic iron) and its regulatory pathways was analyzed by real-time PCR and Western blot. **Results:** Fibrosis developed around the central vein from week 5, and became more extensive with pseudolobule formation from week 12. Advanced cirrhosis was observed from week 15. Serum and liver iron increased in parallel with the progression of cirrhosis. Until week 12, hepatic expression of hepcidin mRNA was upregulated, consistent with the activation of IL6-Stat3 pathway (an inducer of hepcidin transcription). However, from week 15, the hepcidin induction and IL6-Stat3 pathway were inactivated. **Conclusion:** Impaired hepcidin regulation is responsible for the iron overload in the advanced stage of cirrhosis. **Impact statement:** Hepatic iron overload can be involved in the progression of chemically-induced liver cirrhosis.



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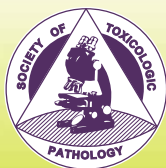
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Congenital Intrathoracic Left Kidney in a Cynomolgus Monkey

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Repeated Subcutaneous Dose Toxicity Studies of Iron Oxide-Zinc Oxide Core-Shell Nanoparticles in C57BL/6 Mice

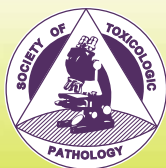
Category: General Pathology/Toxicologic Pathology

Jeong-Hwan Che¹, Jung-Hee Yoon¹, Byeong-Cheol Kang¹, Nam-Hyuk Cho², Seung Hyeok Seok², Young Keun Kim³

¹*Biomedical Research Institute, Seoul National University Hospital, Seoul, Republic of Korea,*

²*Department of Microbiology and Immunology, Seoul National University College of Medicine, Seoul, Republic of Korea,* ³*Department of Materials Science and Engineering and Pioneer Research Center for Biomedical Nanocrystals, Korea University, Seoul, Republic of Korea*

Nanoparticles have been developed as carriers for antigen delivery in the biomedical field. Recently, iron oxide-zinc oxide core-shell nanoparticles (FeZn-NP) were developed as an antigen carrier delivered into dendritic cells. However, the systemic toxicity information of the FeZn-NP was limited. This study was conducted to evaluate the repeated dose toxicity of FeZn-NP after subcutaneous administration. A total of 52 female mice were divided into n=13/group, animals were treated subcutaneously weekly with FeZn-NP doses ranging from 0, 4, 20, and 200 mg/kg B.W. for 28 days. No test article-related effects were observed in a number of parameters including mortality, clinical observations, body weight changes, food and water consumption, hematology, serum biochemistry, and organ weights. However, chronic granulomatous inflammation at subcutaneous injection sites in all treated groups was observed. Biodistribution of FeZn-NP in organs was also examined using inductively coupled plasma atomic emission spectrophotometer (ICP-AES). Taken together, FeZn-NP did not show systemic toxicity up to 200 mg/kg under the conditions of this study.



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Anti-Apoptotic Potential of Herbal Plant Extract in Rats with Cardiomyopathy

Category: General Pathology/Toxicologic Pathology

Harbir Kaur^{1,2}, Gurpreet Singh², Maninder Singh², Ankur Bansal²

¹School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences and Technology, Makhnumajra, Baddi, Distt. Solan, HP, India, ²Department of Pharmaceutics, Rayat Institute of Pharmacy, Railmagra, Distt. SBS Nagar, Punjab, India, ³Ranbaxy Laboratories, Ltd., Mohali, India

Introduction: Cardiomyocyte apoptosis in heart failure has been the topic of research in many recent studies. In the present investigation, the potential cardioprotective effect of gymnemic acid phospholipid complex (GPC) on myocardial apoptosis and cardiac function was studied in doxorubicin (DOX)-induced cardiomyopathy model in rats. **Experimental Design and Methods:** 5 Groups of male Wistar albino rats included Vehicle Control, Pathogenic Control, GPC 50, GPC 100 and Phospholipid per se.

Cardiomyopathy was induced in rats with a single dose of doxorubicin (30 mg/kg/i.p.). **Results:** Pre-treatment with GPC significantly reduced doxorubicin-induced cardiac toxicity, including improvement of hemodynamic variables (systolic, diastolic, mean arterial pressure and heart rate) and heart weight to body weight ratio, decreased serum Ca²⁺ level and lactate dehydrogenase (LDH) level, myocardial caspase-3 levels, increased Na⁺/K⁺ ATPase levels and decreased myocardial tissue thio barbituric acid reactive substances (TBARS) levels and elevated antioxidant enzymes reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) as compared to pathogenic control group. **Conclusions:** Anti-apoptotic effect of GPC was verified by prevention of internucleosomal DNA laddering on agarose gel electrophoresis and attenuation of histopathological perturbations by doxorubicin. **Impact Statement:** These observations demonstrate that GPC might serve as a cardioprotective formulation in doxorubicin-induced cardiomyopathy in rats.



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Artifactual Positive Urine Reagent Test Strip Reactions Caused by Common Contaminants in Laboratory-Housed Nonhuman Primates and Beagle Dogs

Category: General Pathology/Toxicologic Pathology

William Siska¹, Dennis Meyer¹, A. Eric Schultze², Catherine Brandoff¹, Jamie West¹, Steven Wirth¹
¹*Charles River Laboratories, Reno, NV, USA*, ²*Department of Pathology, Lilly Research Laboratories, Indianapolis, IN, USA*

Introduction: A high incidence of positive urine reagent test strip blood reactions was observed in untreated laboratory-housed nonhuman primates (NHP) and beagle dogs (BD). We believed that exposure of urine to cage pan contaminants was likely causative of preanalytical interference and investigated all urine reagent test strip assays (RTSA).

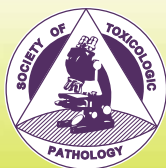
Experimental Design: Distilled water was exposed to potential cage pan contaminants and tested using RTSA.

Materials and Methods: Distilled water was placed in stainless steel cage pans previously cleaned with one of six different procedures. Distilled water was mixed with one of the following in 50 mL conical tubes to achieve liquid consistency - NHP or BD feces, commercially formulated diets, or dietary enrichments (vegetables, fruits, and various treats). Three to 10 aliquots were collected for each experiment after 1 hour, tested with the RTSA and graded according to manufacturer instructions.

Results: Artifactual positive blood reactions were associated with samples contaminated with NHP and BD feces, formulated diets, dietary enrichments, and 2 of 6 cleaning procedures. Contamination with both NHP and BD feces was associated with the strongest positive blood reactions. Positive reactions for other RTSA occurred in highest incidence with dietary enrichments.

Conclusions: We identified a high incidence of artifactual positive RTSA reactions associated with common urine contaminants in NHP and BD used in preclinical toxicology studies.

Impact Statement: These findings add cautionary perspective to the liability and limitations of urine reagent test strip assays as markers of nephrotoxicity in preclinical studies.



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Short-Term Carcinogenic Screening Study Using a Limited Number of Tg-rasH2 Mice

Category: General Pathology/Toxicologic Pathology

Yutaka Nakanishi¹, Satoshi Tsuji¹, Masaki Wakamatsu¹, Hironori Takagi¹, Minoru Sasaki¹, Shunsuke Tsutsumi¹, Yasushi Sato¹

¹*Drug Safety and Pharmacokinetics Laboratories, Taisho Pharmaceutical Co., Ltd., Saitama, Japan*

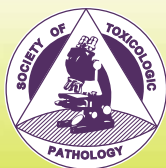
Introduction: We evaluated histopathological changes in CB6F1-TgrasH2 (Tg-rasH2) mice treated with mutagenic or non-mutagenic carcinogen in 4- and 26-week carcinogenic screening studies using a limited number of animals.

Experimental Design and Method: A mutagenic carcinogen, Urethane (UR) at 300 mg/kg/day was administered to Tg-rasH2 male mice and non-transgenic CB6F1 littermates (Non-Tg) male mice for 4 weeks (Week 4). In addition to the evaluation of Week 4, the non-treatment period was set for 22 weeks (Week 26). A non-mutagenic carcinogen, N-methylolacrylamide (NMA) at 1000 ppm was administered to Tg-rasH2 and Non-Tg mice for 4 and 26 weeks (Week 4 and 26). Three males were sacrificed in Week 4 and five males were sacrificed in Week 26, and histopathological examination was performed in all groups.

Results: In Week 4, pulmonary adenomas were noted in the UR-treated Tg-rasH2 mice. In Week 26, pulmonary hyperplasia, adenomas or carcinomas, splenic hemangiosarcomas and retinal atrophy in the eyeball were noted in the UR-treated Tg-rasH2 mice, and pulmonary hyperplasia, adenomas or carcinomas and tubular atrophy in the testis were noted in the NMA-treated Tg-rasH2 mice. These neoplastic changes were more frequently observed in the Tg-rasH2 mice than in the Non-Tg mice.

Conclusion: Carcinogenic potential of UR was detected in Week 4 and 26, while carcinogenic potential of NMA was detected in Week 26 but not Week 4.

Impact statement: The short-term carcinogenic screening study using a limited number of Tg-rasH2 mice could help detecting tumorigenic potential of chemicals.



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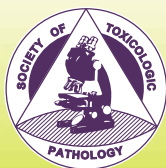
Comparison of Historical Control Parameters Derived from Subchronic Studies Conducted in CD®IGS Rats Fed 5002 or 5CR4 Certified Diets

Category: General Pathology/Toxicologic Pathology

Mark Morse¹, Binod Jacob¹, Aaron Sargeant¹

¹Charles River Laboratories, Spencerville, OH, USA

LabDiet certified rodent 5002 and 5CR4 diets are two diets used in GLP toxicology studies. One of the major differences between the two diets is the protein content (5002: 20% protein; 5CR4: 14% protein). To assess possible differences in the effects of these diets on toxicologic endpoints, historical control parameters from 28-day and 90-day studies conducted within the last 5 years in CD®IGS rats (CrI: CD(SD)) were compared for each diet. Among the parameters evaluated were body weights, food consumption, hematology parameters, coagulation parameters, clinical chemistry parameters, organ weights, and histopathology findings in selected tissues. There were no notable differences in body weights, body weight gain, or food consumption between the two diets. Higher levels of triglycerides and cholesterol were among the few differences in clinical pathology parameters observed in male and female rats fed 5CR4 diet when compared to rats fed 5002 diet, which may be related to a slightly higher fat content in the 5CR4 diet. Kidney weights were approximately 10% greater in male and female rats fed 5002 diet when compared to rats fed 5CR4 diet, but there were no microscopic correlates for this apparent organ weight difference. There were no notable differences in histopathology findings in the liver, lung, or kidneys in rats fed 5002 diet or 5CR4 diet. Overall, there were very few differences in historical control parameters detected in rats fed 5CR4 diet in comparison to 5002 diet, and the two diets are quite comparable when used in subchronic studies in CD®IGS rats.



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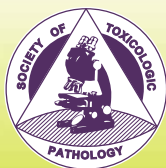
Minocycline, a Putative Neuroprotectant, Co-Administered with Doxorubicin-Cyclophosphamide Chemotherapy in a Xenograft Model of Triple-Negative Breast Cancer

Category: General Pathology/Toxicologic Pathology

Lauren Himmel¹, Maryam Lustberg¹, A. Courtney DeVries¹, Charles Shapiro¹, Ching-Shih Chen¹, Samuel Kulp¹

¹*The Ohio State University, Columbus, OH, USA*

Introduction: Minocycline is purported to have neuroprotective properties in experimental models of some human neurologic diseases, and has therefore been identified as a putative neuroprotectant for chemotherapy-induced cognitive impairment in breast cancer patients. However, because its mechanism of action is believed to be mediated through anti-inflammatory, anti-apoptotic, and anti-oxidant pathways, co-administration of minocycline with chemotherapeutic agents could reduce the efficacy of anticancer drugs. The objective of this study is to evaluate the effect of minocycline on the activity of the AC chemotherapeutic regimen (Adriamycin [doxorubicin], Cytoxan [cyclophosphamide]) in in vitro and in vivo models of triple-negative breast cancer (TNBC). **Methods and Materials:** Clonogenic and MTT assays were used to assess survival and viability in two TNBC cell lines treated with increasing concentrations of AC in the presence or absence of minocycline. Biomarkers of apoptosis, cell stress, and DNA damage were evaluated by western blot. The in vivo effects of AC and minocycline, each alone and in combination, were assessed in a xenograft model of TNBC in female athymic nude mice by weekly tumor volume measurement, body and organ weight measurement, and histopathology. Immunohistochemistry was used to characterize apoptosis and proliferation in the xenografts. **Results:** Data from these in vitro and in vivo studies demonstrate that minocycline does not diminish the cytotoxic and tumor-suppressive effects of this chemotherapeutic drug combination. **Conclusion:** We suggest that minocycline may be useful clinically for its reported neuroprotective activity in breast cancer patients receiving AC without loss of efficacy of these cytotoxic drugs.



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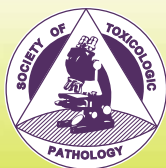
Conditioning Agents for Gene Therapy: Busulfan vs. Irradiation—Common Histopathology Findings in Mice Used in Preclinical Studies.

Category: General Pathology/Toxicologic Pathology

Franck Chanut¹, Francesca Sanvito³, Giuliana Ferrari², Patrizia Cristofori¹

¹GlaxoSmithKline David Jack Centre for R&D, Ware, UK, ²HSR-TIGET Ospedale San Raffaele, Milan, Italy, ³Pathology Unit, San Raffaele Scientific Institute, Milan, Italy

Introduction: Conditioning regimens must induce a sufficient myeloablation to allow engraftment of autologous or allogeneic haematopoietic stem cell transplantation (HSCT) used to treat genetic disorders by gene therapy, and must be sufficiently immunosuppressive to overcome graft rejection of the donor stem cells. In addition, the agents must be well tolerated in the mouse models used for the preclinical studies. **Materials and methods:** We are presenting the major effects of Busulfan and Irradiation observed in mouse models of human diseases used in preclinical toxicity studies that are performed to support the first time in human clinical studies using allogeneic HSCT. **Experimental design:** Mice were pre-treated with busulfan or irradiation to obtain myeloablation and injected intravenously with transduced cells or Mock-transduced cells. Control group included untreated age-matched mutant mice. Recipient and control mice were followed up for 12 months after transplantation and euthanized at the end of this period. **Results:** Besides the haemopoietic system changes, the most commonly affected tissues with both agents were the eyes and the reproductive system, resulting in bilateral lens degeneration and testicular and ovarian atrophy, respectively. In these studies, there was no evidence of treatment-induced tumors. **Conclusion:** Both conditioning agents showed similar histopathology findings and are suitable for use in preclinical safety assessment of gene therapy. **Impact statement:** The study shows that both busulfan and irradiation can be used as conditioning agents in mouse models of human diseases.



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Sex Differences and Litter-Based Effects on Hematologic Parameters from 28-Day Old Harlan Sprague Dawley (Hsd:Sprague Dawley SD) Rats

Category: General Pathology/Toxicologic Pathology

Michelle Cora¹, Greg Travlos¹, Grace Kissling¹, Matt Stout¹, Angela King-Herbert¹, Molly Vallant¹, Chad Blystone¹

¹National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

Introduction: Some National Toxicology Program toxicity studies use a gestational day 6 to postnatal day (PND) 28 exposure paradigm to evaluate potential developmental toxicity. The weaning time point (PND28) could be an opportunity to investigate hematological effects from early life exposure. **Methods:** To investigate feasibility and parameter analysis at this time point, blood was collected from 60 male and 60 female Harlan Sprague Dawley PND28 pups from 15 litters (1-5 pups/sex/litter) and placed in tubes containing ethylenediaminetetraacetic acid; 16 samples were not used due to the presence of blood clots. A standard complete blood count was performed. Mixed effects ANOVAs were used to investigate sex differences and litter-based effects on hematological parameters. **Results:** At PND28, 5/12 parameters were different ($p \leq 0.05$) between males and females; all differences were consistent across litters. Additionally, significant litter effects were observed in 10/12 and 7/12 parameters in males and females, respectively. The coefficients of variations (CV; adjusted for litter) for the erythron were low (1.1% – 6.5%), while the CVs for the leukon were higher (12% – 30%). **Conclusion:** Significant differences between the sexes were observed in almost half of the hematologic parameters at this age and the majority of parameters demonstrated litter effects. For each sex, the erythron CVs were low, indicating low variability within these parameters of PND28 pups. The higher leukon CVs were not unexpected. **Impact Statement:** Analysis of hematologic parameters in weanling (PND28) rats to test for potential developmental effects is feasible and should incorporate the effects of sex and litter.



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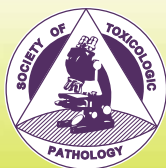
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Common Bile Duct Injury in Wistar Rats Induced by Sorafenib Tosylate

Category: General Pathology/Toxicologic Pathology

Ke Chen^{1,2}, yuanyuan Deng^{1,2}, fen Wang^{1,2}, hongxia Li^{1,2}, xiaobo Cen^{1,2}, chunyan Hu^{1,2}, Peter Mann³
¹National Chengdu Center for Safety Evaluation, West China Hospital, Chengdu, Sichuan, China, ²West China Frontier Pharma Tech Co., Ltd., Chengdu, Sichuan, China, ³EPL Northwest, Seattle, WA, USA

Introduction: Sorafenib tosylate is a novel multi-target anticancer drug, which is mainly metabolized by the liver and eliminated in the feces. We investigated the common bile duct injury caused by Sorafenib tosylate via biliary excretion into the intestine. Experimental Design: Male Wistar rats were administered Sorafenib tosylate by oral gavage at 120 mg/kg/day, 3, 7, 14 or 17 consecutive days. In addition, groups of animals dosed for 17 days were allowed to recover for 7, 14, 28 or 56-days. There were 6 rats in each dose and recovery group. Methods: The lower part of the common bile duct (the intramural duodenal segment) was excised and fixed. H&E staining were performed for morphology examination. Results: Common bile duct lesions were first noted after 14 days dosing, but most severe lesions appeared in the 7-day recovery phase after 17 days dosing. The mild injury consisted of mucosal atrophy; more severe injury was characterized by erosion or ulceration and epithelial hyperplasia. After 14, 28, 56 day's recovery, gradual regeneration and repair of the epithelium and stroma resulted in mucosal thickening. These histologic changes correlated with the gross findings that consisted of enlargement and/or discoloration (yellow-green) of the common bile duct. Conclusion: Wistar rats administered Sorafenib tosylate by oral gavage at 120 mg/kg/day once daily for 14 days caused common bile duct injury. After 14 days recovery, these changes have begun to reverse. Impact statement: This study examined the side effects of Sorafenib tosylate on the common bile duct and suggests the possibility of an adverse reaction of the hepatobiliary system in the clinical application.



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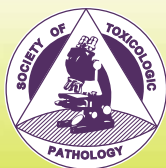
Clinical Pathology Reference Intervals for Healthy Göttingen Minipigs Based on Tolerance Intervals

Category: General Pathology/Toxicologic Pathology

Hala Willis¹, Jodi Boysza¹, Alex Rodriguez¹, Vicky Satterfield¹, Carie Kimbrough¹, Holly Jordan¹

¹GlaxoSmithKline, Research Triangle Park, NC, USA

Objective: Because there is limited information on clinical pathology endpoints in Göttingen minipigs, we generated in-house hematology, clinical chemistry and coagulation reference intervals based on 95% nonparametric tolerance intervals, i.e., ranges with 95% confidence that 95% of data falls within the calculated interval. **Experimental Design and Methods:** Untreated 4m-12m male (n=13) and female (n=16) Göttingen minipigs (Marshall Farms) were acclimated (>5w) prior to sampling. Animals were fasted overnight and samples collected under manual restraint from the cranial vena cava. For hematology, blood was collected into citrate-theophylline, adenosine dipyridamole (for platelet counts to minimize clumping) and potassium ethylenediaminetetraacetic acid for all other hematology parameters. Serum separator tubes were used for clinical chemistry and cardiac troponin I (cTPNI) samples. Prothrombin time (PT) and fibrinogen were obtained using 3.2% sodium citrate. Hematology samples were analyzed within 4h on the Advia 2120[®] analyzer. Serum and plasma tubes were spun (room temp, 3500rpm, 10 min) and samples stored at -80°C before analysis on the Olympus AU640^e analyzer[®] and the STA Compact Analyzer[®]. cTPNI was measured on the Advia Centaur CP[®]. Upper and lower tolerance levels were calculated using an in-house SAS program. Means and standard deviations were also calculated. **Results:** Reference 95% tolerance intervals were determined for routine hematology and clinical chemistry endpoints, cTPNI, PT and fibrinogen. Results for male and female minipigs were generally similar. **Conclusion and impact statement:** Göttingen minipig-specific reference tolerance intervals were generated and tabulated for subsequent use in interpretation of clinical pathology endpoints in our toxicology studies.



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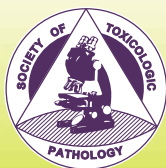
Subchronic Inhalation Exposure of Rats to Libby Amphibole and Amosite Asbestos: Effects at 18 Months Post Exposure

Category: General Pathology/Toxicologic Pathology

Gabrielle A. Willson¹, Darol E. Dodd², Kay Roberts², Henry G. Wall¹, Stephen H. Gavett³

¹Experimental Pathology Laboratories, Inc., Research Triangle Park, NC, USA, ²The Hamner Institutes for Health Sciences, Research Triangle Park, NC, USA, ³US EPA, Research Triangle Park, NC, USA

Increased asbestosis, lung cancer, and mesothelioma rates are evident after exposures to Libby amphibole (LA). To support dosimetry model development and compare potency, a subchronic nose-only inhalation study (6 hr/d, 5 d/wk, 13 wk) was conducted in male F344 rats. Rats were exposed to air (control), LA (LO, MED, HI; 1.01, 3.32, 10.06 mg/m³; 159, 693, 1522 fibers/cc), or amosite (AM; 3.34 mg/m³; 230 f/cc). Toxicity endpoints, pathology, and fiber burden evaluation were determined 18 mo post-exposure. Fiber exposure had no effect on survival. Mononuclear cell leukemia was the main cause of death prior to scheduled necropsy in all groups except the LA 3.3 group. BAL cell numbers, LDH, and protein in AM and LA groups were not statistically different from controls (n=8 rats/group), indicating resolution of earlier inflammation. Histopathology of the left lung, trachea, sternum, pleura, epididymis and testes, and gross tissue lesions was conducted on 50 rats/group. Alveolus inflammation, pleural fibrosis, lung interstitial fibrosis, and foreign bodies were noted in all fiber-exposed groups. A greater incidence of chronic tracheal inflammation was noted in the LA groups. Alveolar bronchiolar adenoma occurred in 2 rats in each of the AM, MED LA, and HI LA groups, and 1 alveolar bronchiolar carcinoma was observed in the HI LA group. No pleural mesotheliomas were observed in any group. In conclusion, both AM and LA induced dose-related lung fibrotic responses; tumor incidences were apparently increased but not beyond historical control ranges. (This abstract does not represent US EPA policy.)



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A 21st Century Roadmap for Human Health Risk Assessment

Category: General Pathology/Toxicologic Pathology

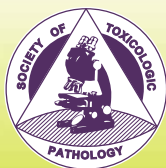
Douglas Wolf¹, Ammie Bachmann², Alan Boobis³, Samuel Cohen⁴, Michael Dellarco⁵, Ian Dewhurst⁶, John Doe⁷, Nancy Doerrer⁸, Angelo Moretto⁹, Timothy Pastoor¹, Richard Phillips², J. Craig Rowlands¹⁰, Jennifer Tanir⁸, Michelle Embry⁸

¹Syngenta, Ltd., Greensboro, NC, USA, ²ExxonMobil Biomedical Sciences, Inc., Annandale, NJ, USA,

³Imperial College London, London, UK, ⁴University of Nebraska Medical Center, Omaha, NE, USA,

⁵National Institute of Child Health and Human Development, Bethesda, MD, USA, ⁶UK Chemicals Regulation Directorate, York, UK, ⁷Parker Doe Partnership LLP, Chesire, UK, ⁸ILSI Health and Environmental Sciences Institute, Washington, DC, USA, ⁹University of Milan, Milan, Italy, ¹⁰The Dow Chemical Company, Midland, MI, USA

The ILSI Health and Environmental Sciences Institute (HESI) Risk Assessment in the 21st Century (RISK21) project was initiated to develop a scientific, transparent, and efficient approach to the evolving world of human health risk assessment. RISK21 developed a framework that reconsiders the way chemical risk assessment information is obtained and used. It is a problem formulation-based, exposure driven, tiered data acquisition approach that allows an informed decision on human health safety to be made when sufficient evidence is available. The RISK21 approach maximizes the ability to inform decisions and optimize resource usage. Two case studies were developed to illustrate these principles. The first example identified testing needs for a new 'nth' in class pesticide to be used in mosquito netting for malaria prevention, and illustrated how existing information from other pesticides in the same chemical class and knowledge of use patterns can inform data needs and decision making. In the second example, a large number of chemicals which might be present in drinking water were prioritized and evaluated to determine which are of highest potential concern for human health risk assessment. Both case studies also identified key issues and possible approaches to address cumulative risk. The overall goal of these examples was not to make definitive risk assessment determinations but to establish the utility of the RISK21 framework in assessing the value of available information and making decisions about what, if any, additional information is needed to inform a decision.



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Lack of *In Vivo* Genotoxicity and Rat Toxicity of Myricitrin Administered at Dietary Levels up to 5%

Category: General Pathology/Toxicologic Pathology

Shim-mo Hayashi¹, Jeffrey Davis², Cheryl Hobbs², Carol Swartz², Leslie Recio², Robert Maronpot³

¹San-Ei Gen FFI, Inc., Osaka, Japan, ²Integrated Laboratory Systems, Inc., Research Triangle Park, NC, USA, ³Maronpot Consulting, LLC., Raleigh, NC, USA

Introduction: Chinese bayberry (*Mirica rubra* SIEBOLD) extract is used as a food additive and antioxidant in flavor modifiers, snack foods, dairy products, and beverages in Japan. Myricitrin is affirmed as “generally recognized as safe” by the U.S. Flavor and Extract Manufacturer Association. The genotoxic and toxic potential of myricitrin was evaluated in anticipation of a positive safety opinion from JECFA (Joint FAO/WHO Expert Committee on Food Additives), and the eventual global marketing of products containing myricitrin. **Experimental Design:** Myricitrin was evaluated in a bacterial reverse mutation assay, an in vitro micronucleus assay using human TK6 lymphoblast cells, and a 3-day combined micronucleus (peripheral blood) and Comet (liver and duodenum) assay using male B6C3F1 mice.

Myricitrin was also evaluated in a 90-day toxicity study using male and female Sprague Dawley rats. All studies were conducted according to OECD testing guidelines. **Results:** Negative results were observed for myricitrin in both the bacterial mutation and in vitro micronucleus assays. No induction of micronuclei or DNA damage was observed in mice following exposure to myricitrin. There were no adverse clinical or gross observations in rats in the repeat dose study using myricitrin at dietary concentrations up to 5%.

Conclusion/Impact: Our in vivo studies do not provide any evidence of genotoxic potential or toxicity of myricitrin, supporting its safe use in food and beverages.



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Development of Photoaging Model Using UVB and Heat

Category: General Pathology/Toxicologic Pathology

Il-Hong Bae¹, Ji-Hae Lee¹, Dae-Yong Kim¹

¹College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

Introduction: Animal models have been developed to study photoaging. UVB irradiation in dorsal skin of hairless mice is a common method, which induces aggravation of extracellular matrix composition (ECM) and wrinkles. This model, however, requires a long period of over 12 weeks and is often accompanied by inflammation, possibly impeding mechanism study of photoaging.

Experimental design: To draw effective photoaging in shorter study period, we applied UVB and heat simultaneously for 6 weeks.

Methods: Hairless mice were divided into 4 groups; G1 (No UVB), G2 (UVB, 6 weeks), G3 (UVB plus heat, 6 weeks), G4 (UVB, 12 weeks). UVB was irradiated 3 times a week and the intensity were gradually increased from 1 to 4 minimal erythral dose (50mJ/cm²). 45°C heat was given for 15 minutes in the dry chamber.

Results: Macroscopically, UVB-irradiated groups showed an increase of trans-epidermal water loss, skin thickness, erythral level, and coarse wrinkles. G2 and G3 exhibited similar photoaged features, while it was more deteriorated in G4. Histologically, the numbers of sunburn cells and inflammatory cells were observed in G4, which was not apparent in G2 and G3. Abnormal ECM was markedly noticeable in G3 and G4, while G2 did not show any significant change.

Conclusion: 6-week irradiation of UVB and heat induced similar photoaged features of current 12-week methods but lacked robust cutaneous inflammation.

Impact statement: We propose combined actinic and thermal irradiation as alternative method to induce photoaging with advantages of reducing study period and dermal inflammation.



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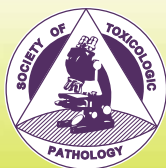
A Decade of Non-Neoplastic Histologic Background Lesions in Göttingen Minipigs at MPI Research

Category: General Pathology/Toxicologic Pathology

Charlotte Hollinger¹, Keith Nelson^{2,1}

¹Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA, ²MPI Research, Mattawan, MI, USA

Introduction: Göttingen minipigs are increasingly promoted for use as non-rodent models in toxicologic studies. Accurate recognition and quantification of spontaneous lesions in animal models improves identification of compound-related findings, and may reveal population-related variation. The purpose of this study was to review and analyze non-neoplastic background lesions occurring in control Göttingen minipigs at MPI Research over ten years. **Methods:** Histologic lesion data were tabulated for 416 animals (205 males and 211 females), from 29 dermal and 11 oral gavage studies ranging from 2 to 36 weeks in duration. Comparisons of lesion prevalence were made across gender and age. **Results:** The most prevalent findings were ovarian mineralization (29.9%), renal mononuclear infiltration (27.2%), testicular hypoplasia/degeneration (24.4%), hepatic mononuclear infiltration (16.6%), renal tubular degeneration/regeneration (16.4%), and prostatic mineralization (15.1%). Renal tubular, glomerular, and mixed inflammatory changes were twice as prevalent in females. Younger animals had 3-fold increases in hepatic mononuclear infiltrates and 4-fold increased prevalence of salivary gland mineralization. Mineralization and mononuclear infiltration were present in a variety of organs in all ages and sexes. Previously reported findings of necrotizing cholecystitis and serous atrophy of fat were not recorded in this population. **Conclusions:** Göttingen minipigs develop a range of spontaneous changes, which may affect analysis of compound-related effects. The prevalence of some lesions varies with age and gender, and there may be inter-population differences potentially related to source or institutional factors. **Impact statement:** This study provides an extensive review of background lesions in the Göttingen minipig at a large preclinical CRO.



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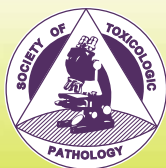
Isoproterenol Induced Skeletal Troponin Elevation in Sprague Dawley Rats

Category: General Pathology/Toxicologic Pathology

Malar Pannirselvam¹, Quincey Simmons¹, Jessica Wan¹, Suzanne Botts¹, David Brewster¹

¹Vertex Pharmaceuticals, Boston, MA, USA

Serum levels of cardiac troponin I and cardiac troponin T are used clinically in the detection of acute myocardial infarction and other cardiac conditions. These along with other cardiac specific biomarkers such as fatty acid-binding proteins have been measured in several experimental models including rats, mice, dogs and non-human primates and have been established as useful biomarkers of cardiac injury in rats and dogs. The objective of the present study was to establish the specificity of isoproterenol induced cardiotoxicity using a rat muscle injury panel and histopathology in male Sprague Dawley rats. Rats were injected with saline, 1 ml/kg or isoproterenol, 4 mg/kg, subcutaneously. Four and 24 hours post-isoproterenol injection, blood samples were collected for troponin levels and 24h heart and skeletal muscle were harvested for histopathologic examination. Isoproterenol injection induced a significant increase in the cardiac troponin I levels, Fatty acid binding protein and myosin light chain at 4h and correlated to ventricular damage on histopathology evident at 24h. There was also a significant increase in the skeletal muscle troponin levels at 4h post isoproterenol administration. At 24h routine H&E staining and staining with pentachrome showed no histologic evidence of inflammation in sections of skeletal muscle; however, the epimyseum was slightly more cellular. Immunohistochemistry with CD68, a marker of macrophage activation, showed an increase in stain uptake by macrophages in the epimyseum suggesting that macrophages were in an activated state. In summary, isoproterenol at 4 mg/kg induced a non-specific muscle injury in Sprague Dawley rats.



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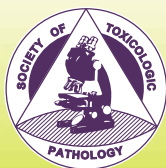
Effects of Perinatal Exposure to Single or Mixed Fungicides on the Female Rat Reproductive System

Category: General Pathology/Toxicologic Pathology

Viviane Pascotto¹, Carla Franchi¹, Janete Franci², Ruiether Carolino¹, João Lauro Camargo¹

¹São Paulo State University UNESP Medical School, Botucatu, Brazil, ²School of Dentistry of Ribeirão Preto, University of São Paulo, USP, Ribeirão Preto, Brazil

Introduction: Derived triazoles and imidazoles fungicides interfere with steroid hormones homeostasis leading to adverse effects during intrauterine and postnatal life. Azole fungicides were evaluated for effects on the female reproductive system. **Methods:** Pregnant rats were allocated to four groups fed experimental diets from the 9th gestational day (GD9) to the end of lactation (postnatal day 22, PND22): G1- control, G2- 150ppm prochloraz, G3- 500ppm propiconazole and G4- mixture of both pesticides at the respective levels. After weaning, female offspring received plain diet until PND140 when they were sacrificed in metestrus. **Results:** Neonates did not show clinical or physical signs of toxicity. There were no differences among the groups regarding AGDs and BWs at PND2. Female offspring exposed to prochloraz or propiconazole showed delayed vaginal opening ($p < 0.01$ or $p < 0.05$, respectively) when compared to the control or to animals treated with the fungicide mixture ($p < 0.01$). Final BWs and relative weights of liver, spleen, uterus and ovary did not show differences from the control. Testosterone, estradiol and progesterone levels were not affected, but LH levels were higher in the fungicide-treated groups (prochloraz, $p < 0.001$; propiconazole, $p < 0.01$; fungicide mixture, $p < 0.01$). FSH levels were significantly elevated ($p < 0.001$) in the mixture-exposed group when compared to the control or to the groups treated with individual fungicides. **Conclusion:** Individual fungicides delayed the completion of sexual maturation and both individual and mixtures of fungicides altered gonadotropins, especially LH. **Impact statement:** Exposure to azole fungicides impacted negatively on the reproductive system of female rats exposed in utero and during lactation.



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Lysosomal Drug Accumulation and Precipitation in Rats Seen with an Amphoteric Compound

Category: General Pathology/Toxicologic Pathology

Barbara Lenz¹, Urs Niederhauser¹, Holger Fischer¹, Rodolfo Gasser¹, Claus Riemer¹, Alexander Flohr¹, Thomas Weiser¹, Thomas Singer¹, Nicole Wyttenbach¹, Mudher Albassam¹, Monira Siam¹
¹*Hoffmann-La Roche, Basel, Switzerland*

Introduction: Sequestering of weakly basic drugs in acidic organelles is a well-known phenomenon and particularly seen with phospholipidosis. An amphoteric compound bearing weakly basic and anionic moieties, with good solubility at physiological pH was negative for phospholipidosis *in silico* and only weakly positive *in vitro*. Widespread crystalline tissue deposition was noted in rat studies which triggered further investigations.

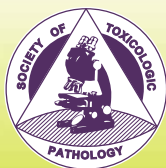
Experimental Design: 2-week and 4-week oral rat toxicity studies were performed.

Methods: Investigations included histopathology, CD68/Lamp-2 immunostaining, electron microscopy, frozen tissue section examination, Raman microspectroscopy/IR-spectroscopy of crystals and drug concentration analysis in plasma & bone marrow. A pH partition model for ampholytes was developed to describe compound distribution in plasma, cytosol and lysosomes. The thermodynamic solubility of the compound was measured at pH 5.1.

Results: Foamy/vacuolated macrophages were seen in lungs and lymph nodes and Lamp-2 immunohistochemistry suggested lysosomal accumulation. Additionally there were macrophage aggregates with cytoplasmic membrane bound crystals in multiple tissues which were identified by Raman microspectroscopy/IR-spectroscopy as hydrochloride salt of the parent. High parent concentration in the bone marrow confirmed tissue accumulation. The lysosomal compound concentration was estimated based on physico-chemical properties and plasma exposure at C_{\max} . A good correlation was obtained between precipitates, lysosomal concentration and compound solubility at pH 5.1.

Conclusion: Despite good solubility at physiological pH the amphoteric compound accumulated and subsequently precipitated in lysosomes because of limited solubility in acidic media.

Impact statement: The accumulation potential in lysosomes can be estimated by a simple method based on physico-chemical properties of the compound.



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Mitochondrial Alteration in CD1 Mice Associated with Prenatal Exposures to Low Doses of Perfluorooctanoic Acid (PFOA): A PPAR α -Independent Mode of Action?

Category: General Pathology/Toxicologic Pathology

Erin Quist^{1,2}, Adam Filgo^{1,3}, Connie Cummings⁵, Grace Kissling⁴, Mark Hoenerhoff⁶, Suzanne Fenton¹

¹NTP Laboratory Branch, NTP, NIEHS, NIH, DHHS, Research Triangle Park, NC, USA, ²Comparative Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA, ³Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC, USA, ⁴Biostatistics Branch, DIR, NIEHS, NIH, DHHS, Research Triangle Park, NC, USA, ⁵UltraPath Imaging, Durham, NC, USA, ⁶Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI, USA

Introduction: Perfluorooctanoic acid (PFOA) is a perfluoroalkyl acid primarily used as an industrial surfactant. It persists in the environment and has been linked to potentially toxic and/or carcinogenic effects in animals and people. As a known activator of peroxisome proliferator-activated receptors (PPARs), PFOA can cause alterations that lead to defects in fatty acid oxidation, lipid transport, and inflammation. **Experimental Design:** Pregnant CD-1 mice were orally gavaged with 0, 0.01, 0.1, 0.3 and 1 mg PFOA/kg body weight from gestation days (GD) 0 through 17. At postnatal day (PND) 35, offspring were additionally subdivided into 3 dietary groups for a high-fat diet challenge: 1) high fat diet- fasted 2) high fat diet- non-fasted and 3) control diet. **Methods:** Tissues were collected on PND 21 and 91 and routinely processed for histological evaluation. Frozen sections of liver were also collected for transmission electron microscopy (TEM). **Results:** On PND 21, histopathologic changes in the liver of offspring included hepatocellular hypertrophy and peri-portal inflammation that increased in severity by PND 91. TEM of liver from PND 91 mice revealed PFOA-induced cellular damage and mitochondrial abnormalities with no evidence of peroxisome proliferation. Within hypertrophied hepatocytes, mitochondria were not only increased in number, but also exhibited altered morphologies suggestive of increased and/or uncontrolled fission and fusion reactions. **Conclusion:** We conclude that prenatal exposures to PFOA at a dose of 1 mg/kg induced hepatocellular hypertrophy in CD1 mice due to mitochondrial proliferation. **Impact Statement:** The mechanism of PFOA-induced hypertrophy appears to be independent of PPAR α activation.



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Histologic Findings Associated with Chronic Femoral Artery Implantation of a Miniature Telemetry Blood Pressure Transmitter in Cynomolgus Monkeys

Category: General Pathology/Toxicologic Pathology

William Meier¹, Laura Kreckler¹, John Kremer¹, Mike Foley¹, Mark Holbrook², Niraj Tripathi¹, Alok Sharma¹, Emily Meseck¹

¹Covance Laboratories, Madison, WI, USA, ²Covance Laboratories, Harrogate, UK

Introduction: The use of jacketed external telemetry (JET) in general toxicology studies is an important tool for efficient use of research animals and to collect and assess large quantities of cardiovascular data absent the effects of manipulation and restraint. Validation of JET systems often fails to mimic the rigors of a standard repeat-dose toxicology study in terms of animal numbers, duration, and relevant use of physiologically active test articles for real-world interpretation of end points. **Experimental Design:** The purpose of this study was to evaluate the tolerability, functionality, and sensitivity of a minimally invasive implanted miniature telemetry blood pressure transmitter in conjunction with electrocardiographic measurements using JET in nonhuman primates administered four test articles used as positive controls. **Methods:** Pathology findings were assessed in relation to chronic femoral artery implantation of a miniature telemetry blood pressure transmitter in 36 male cynomolgus monkeys for up to 45 weeks post-implant. **Results:** Microscopic findings were consistent with the expected findings of an implanted device with an indwelling arterial catheter and similar to tissue responses often seen with other long-term intravascular catheter systems. **Conclusion:** Data collection using miniature telemetry blood pressure transmitters for up to 45 weeks did not result in pathology findings that would prevent their use in general toxicology studies. **Impact Statement:** This approach is expected to allow for the rapid collection and sensitive assessment of electrocardiographic and blood pressure telemetry data and correlation to relevant test article-related physiologic and pathology end points while saving time and overall drug development cost.



Non-Lesions, Misdiagnoses, Missed Diagnoses, and Other Interpretive Challenges in Fish Histopathology Studies: A Guide for Investigators, Authors, Reviewers, and Readers

Category: General Pathology/Toxicologic Pathology

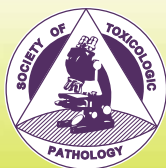
Jeffrey Wolf¹, Wes Baumgartner², Vicki Blazer³, Alvin Camus⁴, Jeffery Engelhardt⁵, John Fournie⁶, Salvatore Frasca⁷, David Groman⁸, Michael Kent⁹, Lester Khoo¹⁰, Jerry Law¹¹, Eric Lombardini¹², Christine Ruehl-Fehlert¹³, Helmut Segner¹⁴, Stephen Smith¹⁵, Jan Spitsbergen¹⁶, Klaus Weber¹⁷, Marilyn Wolfe¹⁸

¹Experimental Pathology Laboratories, Inc., Sterling, VA, USA, ²Department of Pathobiology/Population Medicine, College of Veterinary Medicine, Mississippi State, Mississippi State, MS, USA, ³US Geological Survey, Kearneysville, WV, USA, ⁴Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA, ⁵Experimental Pathology Laboratories, Inc., Camarillo, CA, USA, ⁶US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL, USA, ⁷Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT, USA, ⁸Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, ⁹Departments of Microbiology and Biomedical Sciences, Oregon State University, Corvallis, OR, USA, ¹⁰Mississippi State University, College of Veterinary Medicine, Stoneville, MS, USA, ¹¹Aquatic Ecotoxicology, North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA, ¹²Divisions of Comparative Pathology and Veterinary Medical Research, Department of Veterinary Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, ¹³Bayer HealthCare AG, Wuppertal, Germany, ¹⁴Centre for Fish and Wildlife Health, University of Bern, Bern, Switzerland, ¹⁵Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA, ¹⁶Fish Disease Research Group, Department of Microbiology, Oregon State University, Corvallis, OR, USA, ¹⁷AnaPath GmbH, Oberbuchsitzen, Switzerland, ¹⁸EPL, Inc., Sterling, VA, USA

Introduction: Differentiating salient histopathologic changes from normal anatomic features or tissue artifacts can be especially challenging for the novice fish pathologist. Consequently, findings of questionable accuracy may be reported inadvertently. The objectives of this project were to identify specific morphologic findings in commonly examined fish tissues that are frequently either misdiagnosed or underdiagnosed, and to illustrate such findings through the use of photomicrographic examples.

Experimental Design and Methods: A number of highly-trained, veteran fish pathologists were tasked with assembling lists of histopathologic diagnoses that often appeared questionable based on evaluations of published morphologic descriptions and figure illustrations. For the current project, photomicrographic examples of normal and abnormal specimens were acquired from the personal slide collections of the authors, or obtained by permission from prior studies. **Results:** Histopathologic findings that appeared to be commonly over-diagnosed or misdiagnosed in the literature included nine types of gill diagnoses, six kidney diagnoses, four liver diagnoses, and five additional diagnoses in various other tissues.

Additionally, the authors identified nine types of findings that tend to be under-reported. **Conclusion:** Histopathology continues to be a valuable tool for investigating the morphologic features and extent of both naturally-occurring and experimentally-induced disease. The authors describe practical measures that can be instituted to safeguard against the publication of dubious histopathologic results. **Impact statement:** The fundamental goal of this effort is to elevate the science and practice of fish histopathology, which has become an increasingly important discipline in fields that include basic biomedical research, aquaculture, environmental resource management, and ecotoxicology.



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Location Matters—the Behavior of Human Retinoblastoma Cells Is Dependent on the Site of Implantation

Category: General Pathology/Toxicologic Pathology

Julia Baker¹, Shawna Jackman²

¹Charles River Pathology Associates, Frederick, MD, USA, ²Charles River Preclinical Services, Horsham, PA, USA

Introduction: Human retinoblastoma cells are not uncommonly used as a positive control article in intraocular stem cell safety studies. During a pilot study to compare the suitability of two different strains of immunocompromised mouse for subretinal stem cell injection, 3/6 female NSG mice inadvertently received cells injected in the vitreous. The behavior of the retinoblastoma cells at this site was very different to the behavior seen in the mice injected subretinally.

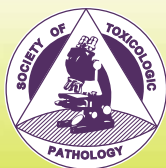
Experimental Design: A pilot biodistribution study to compare the suitability of pigmented Crl:NIH-LystbgFoxn1nuBtkid homozygous nude (NIH-III) mice with albino NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice for an upcoming preclinical safety study looking at a subretinal stem cell therapeutic product included a positive control group using human retinoblastoma cells. Twelve NSG mice (6 males/6 females) and twelve NIH-III mice (6 males/6 females) were assigned to this group.

Methods: Mice were injected at 3–4 weeks of age and were maintained for 28 days post-injection. Eyes from positive control animals that survived to the scheduled day of necropsy were routinely fixed and processed to paraffin, sectioned, and stained with hematoxylin and eosin (HE).

Results: All 18 mice administered human retinoblastoma cells subretinally developed locally invasive masses within the injected eye. Cells that had been inadvertently deposited in the vitreous (3 mice) failed to develop any characteristics suggestive of neoplasia.

Conclusion: Retinoblastoma cells are highly sensitive to their environment, and their behavior may be modified by incorrect placement.

Impact Statement: These results emphasize the importance of accurate administration in safety studies pertaining to stem cells.



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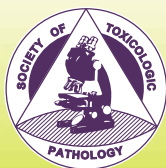
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Quantitative Evaluation of Drug-Induced Microvascular Constriction in Mice Kidney Using a Novel Tool for 3D Geometrical Analysis of *Ex Vivo* Organ Vasculature

Category: New Technologies

Raul Brauner², Yuval Ramot¹, Kongbin Kang^{2,4}, John V. Heymach³, Stacia Furtado^{2,4}, Abraham Nyska⁵
¹Hadassah – Hebrew University Medical Center, Jerusalem, Israel, ²Bio-Tree Systems, Inc., Framingham, MA, USA, ³MD Anderson Cancer Center, Houston, TX, USA, ⁴Brown University, Providence, RI, USA, ⁵Toxicologic Pathologist, Timrat, Israel

Introduction: The analysis of organ vasculature, and more specifically organ microvasculature, carries special importance for toxicological sciences, and especially for evaluation of drug-induced vascular toxicity. This field presents a special challenge in non-clinical drug safety assessments since there are currently no reliable microvascular toxicity biomarkers. Therefore, we aimed to systematically investigate the use of microvascular 3D geometrical analysis of corrosion casts for evaluation of drug-induced vascular toxicity, utilizing a novel image investigation tool which allows full 3D quantified geometrical analysis of the entire vascular tree structure. **Methods:** Vascular casts of kidneys from control and low- and high-dose ephedrine/caffeine-treated mice were scanned by a micro CT, and images were processed and analyzed using the Vasculomics™ platform. All evaluations were performed on the kidney cortex. **Results:** Treatment resulted in a significant and dose-related reduction in overall micro-vessel density throughout the kidney cortex. This effect was most pronounced for vessels with diameters between 25 to 35µm, and affected mostly vessels located in the superficial part of the kidney cortex. **Conclusions:** The use of 3D analysis tools in drug-induced vascular toxicity studies allows for very high-resolution and characterization of drug effects on the microvasculature, and can be used as a valuable tool in drug safety assessments.



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Significant Lessening of Local Reactions Following Continuous SC Administration of ND0701, a New Apomorphine Formulation for Parkinson's Disease—MRI and Histopathology Studies

Category: New Technologies

Ronit Shaltiel-Karyo¹, Hanita Ovadia¹, Yonit Tzarfati¹, Yael Schiffenbauer², Abraham Nyska³, Oron Yacoby-Zeevi¹

¹NeuroDerm Company, Ness-Ziona, Israel, ²Aspect Imaging, Shoham, Israel, ³Consultant in Toxicologic Pathology, and Tel Aviv University, Timrat, Israel

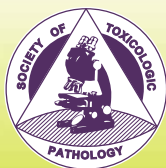
Introduction: Continuous subcutaneous infusion of drug formulations often results in local damage at the administration site. *In vivo* and ex vivo MRI was used to evaluate the local damage of commercial Apo-Go, used as symptomatic therapy in Parkinson's disease, and a newly developed apomorphine formulation ND0701. The results were compared to histopathology.

Experimental Design & Methods: The drug formulations ND0701 and Apo-Go® were administered to domestic pigs by 24 hour continuous subcutaneous infusion. Follow-up of damage at the infusion site was performed using *in vivo* MRI, two and four weeks post drug administration in a Magnetom-C MRI machine (Siemens). Ex vivo MRI was performed thereafter on excised skin tissues, using the novel compact MRI system (Aspect Imaging) followed by histopathology.

Results: *In vivo* MRI showed significantly smaller lesions at the infusion site 2wks post infusion, with almost no damage observed 4wks following 1% ND0701 administration. Damage was clearly evident 4wks following 1% Apo-Go® administration. Ex vivo compact MRI and histopathology revealed severe damage following Apo-Go® injection, consisting of necrosis surrounded by granulomatous inflammation, whereas minor chronic inflammation was observed with 1% ND0701.

Conclusion: While *in vivo* MRI was highly efficient in following the recovery at the injection sites, ex vivo compact MRI allowed quantification of the damage with good correlation to histopathology evaluation. ND0701 was proved to be tolerated much better locally.

Impact statement: MRI was proven useful in evaluating toxicologic damage as a result of subcutaneous drug administrations.



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The Development of Image Analysis Algorithms to Objectively Characterize the Host Tissue Response to an Encapsulated Stem Cell Product

Category: New Technologies

John J O'Neil¹, Allison Rubin², Irina Batushansky², David Young³

¹Janssen Research and Development, LLC., Raritan, NJ, USA, ²Janssen Research and Development, LLC., Springhouse, PA, USA, ³Flagship Biosciences, LLC., Boulder, CO, USA

Introduction: Characterization of the host tissue response to a stem cell based therapy in a preclinical setting will be mandatory prior to first in human studies. Many endpoints associated with the evaluation of host response are subjectively derived based on pathology experience and expertise. Image analysis solutions offer a quantitative assessment of tissue changes and allow a more direct analysis and comparison of endpoints. **Experimental Design:** Two medical device designs containing a stem cell derived therapy implanted subcutaneously in NSG mice (JAX 005557) for 24 weeks were formalin fixed, paraffin embedded, sectioned, H&E stained and scanned to create digital images. **Methods:** Using proprietary algorithms, image analysis solutions were applied to detect and quantify neovascularization, multinucleated giant cells and granulation tissue. Directed solutions were applied to quantitatively measure the endpoints to the region of interest. **Results:** The algorithms developed were able to objectively quantitate the host tissue response including neovascularization and multi-nucleated giant cells that had migrated to the medical device as well as the extent of granulation tissue formed in response to the device. However, there was no statistical difference between the two device designs. **Conclusion:** Although the use of special staining for blood vessels, macrophages, and collagen would provide more comprehensive results, this study demonstrates the ability to objectively quantitate host tissue response to a stem cell derived therapy. **Impact Statement:** Objective quantitative digital image analysis of host tissue response to stem cell therapies will eliminate inherent subjectivity of conventional methods and could lead to standardization across the field.



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The Use of Immunohistochemistry and Image Analysis to Objectively Characterize the Composition of a Stem Cell-Derived Therapy in Preclinical Studies

Category: New Technologies

Jing Ying Ma¹, Allison Rubin², Irina Batushansky², John J O'Neil³

¹Janssen Research and Development, LLC., La Jolla, CA, USA, ²Janssen Research and Development, LLC., Springhouse, PA, USA, ³Janssen Research and Development, LLC., Raritan, NJ, USA

Introduction: The differentiation of pluripotent stem cells prior to implantation will likely result in a heterogeneous cell population consisting of undifferentiated, partially differentiated and fully differentiated cells and as such, may not be clearly identifiable using conventional histological staining techniques following implantation. The use of immunohistochemistry for cell identification and image analysis for graft quantitation will allow for more comprehensive characterization of the stem cell product.

Experimental Design: Human embryonic stem cells were differentiated towards a pancreatic precursor cell population using two differentiation protocols. The differentiated cells were transplanted into SCID-beige mice (TACONIC - CBSCBG) for 24 weeks and were formalin fixed, paraffin embedded and sectioned. **Methods:** IHC double labeling with insulin, glucagon, somatostatin, chromogranin, CK19, Nkx6.1 antibodies and Ki67 was performed. Slides were scanned at 40X using the Hamamatsu whole slide scanner and Visiopharm-software was used for quantitative image analysis. **Results:** The analysis objectively quantitated the percentage of the total cell population positive for the antibodies evaluated, the percentages of the positive cells proliferating, and the percentage of antibody positive proliferating cells.

Conclusion: Although a more extensive antibody panel would be required to account for all of the cells in this stem cell-derived therapy, this study demonstrates the ability to objectively quantitate cell populations that arise from a stem cell derived therapy. **Impact Statement:** Immunohistochemistry followed by objective quantitative digital image analysis allows for the identification and quantitation of differentiated cells within a stem cell-derived therapy which will aid in the safety and efficacy assessment of the transplanted cells.



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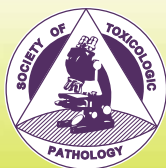
First Time in Man Enabling Study Using Gene Therapy to Treat Beta-Thalassemia: Focus on the Histopathology

Category: New Technologies

Franck Chanut¹, Francesca Sanvito^{3,2}, Francesca Tiboni², Giacomo Mandelli², Maria-Rosa Lidonnici², Paola Albertini², Luigi Naldini², Giuliana Ferrari², Patrizia Cristofori¹

¹GlaxoSmithKline David Jack Centre for R&D, Ware, UK, ²San Raffaele-Telethon Institute for Gene Therapy (TIGET), Milan, Italy, ³Pathology Unit, San Raffaele Scientific Institute, Milan, Italy

Introduction: Beta thalassemia is an inherited blood disorder characterized by a reduced or absent synthesis of β -globin chain that results in insufficient production of hemoglobin A (HbA). Transplantation of autologous, genetically corrected hematopoietic stem/progenitor cells (HSPCs) represents an alternative therapy for patients lacking a suitable bone marrow donor. **Materials and methods:** We used transplantation of murine bone marrow (BM) derived lineage-negative (Lin-) HSPCs transduced with GLOBE.LV (an erythroid-specific lentiviral vector driving the expression the human b-globin) in recipient conditioned C57BL6/Hbb^{th3} mutant (th3/+) mice, representing the homologous disease model of severe thalassemia intermedia in humans. **Experimental design:** Mice (15 animals/sex/group) were pre-treated with busulfan (myeloablation), injected intravenously with GLOBE.LV transduced cells or Mock-transduced cells. Control group included untreated age-matched mutant (th3/+) mice. Recipient and control th3/+ mice were followed up for 12 months and euthanized at the end of this period. **Results:** A high level of engraftment of transduced cells and persistent transgene expression inducing a persistent correction of hematological abnormalities in group 1 mice was observed. The efficacy was also demonstrated microscopically with decreased incidence and severity of the mouse model phenotype-related changes (extramedullary hematopoiesis and erythrophagocytosis). In this study, there was no difference in incidence and type of tumors between groups. **Conclusion:** This study supports the value of using animal models of human disease for both efficacy and safety assessment and shows the efficacy of gene therapy in the murine model of human b-thalassemia.. **Impact statement** The study provides long-term evidence of good safety profile after treatment with GLOBE.LV transduced murine HPSCs.



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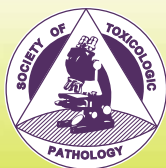
Development of Tissue Image Analysis Tools to Identify Murine Pancreatic Intraepithelial Neoplasia

Category: New Technologies

Famke Aeffner¹, Mirza Peljto¹, Michael O. Ports², Thorsten Hagemann³, G. David Young¹

¹Flagship Biosciences, LLC., Boulder, CO, USA, ²Gilead Sciences, Seattle, WA, USA, ³Barts Cancer Institute, Queen Mary University of London, London, UK

Introduction: Whole tissue image analysis (tIA) was used to identify pancreatic intraepithelial neoplasia (PanIN) lesions in the pancreas of KPC mice (KrasLSL.G12D/+; p53LSL.R172H/+; PdxCre^{tg}/+) and to evaluate cell proliferation within tumor epithelium or tumor microenvironment (TME). **Experimental Design:** Pancreas of nine KPC mice were harvested two weeks following positive identification of primary tumors by ultrasound (2-5mm diameter), formalin fixed, paraffin embedded, and routinely processed. Ki67 expression was examined using immunohistochemistry (IHC). Slides were digitized utilizing Aperio's CS bright-field scanning system. Digital whole-slide images were analyzed with Flagship's proprietary tIA algorithms. **Methods:** Digital images of the IHC slides were manually annotated to isolate PanIN and TME from surrounding tissue. Full spectrum of PanIN stages was present in the cohort. Flagship's CellMapTM 0.7 algorithm and statistical approaches were used for development of tIA techniques to appropriately identify tissue compartments, individual cells and to quantify Ki67 biomarker content. **Results:** CellMapTM algorithm operated efficiently in identifying PanIN and TME cells with a high degree of precision and quantifying Ki67+ nuclear staining in each tissue compartment. **Conclusion:** tIA tools allowed for identification of all grades of PanIN and separate quantification of Ki67 content in tumor and TME. There was no significant difference in Ki67 expression between PanIN and TME. **Impact statement:** A novel tIA strategy was developed to evaluate highly complex and diverse lesion patterns within the murine pancreas. This provides a powerful tool to reliably analyze treatment effects of novel therapeutics that may aim at preventing advancement of disease.



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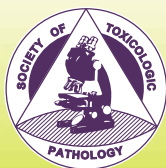
Photodynamic Therapy (PDT) Applied to the Primary Ehrlich Tumor Induces Inhibitory Effects on a Second Implant of the Same Tumor

Category: New Technologies

Murilo Del-Grande¹, Maria Lucia Zaidan Dagli¹

¹*School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil*

Introduction: Photodynamic therapy (PDT) is based on the interaction among light, a photosensitizing agent and the production of reactive oxygen species which cause cell death. We hypothesized that the activation of the innate immune response by PDT could precede an adaptive immune response driven to a second implant of the Ehrlich tumor cells. **Experimental Design:** BALB/c mice received subcutaneous inoculation of the Ehrlich tumor, which was treated with PDT or surgically removed. After 9 days, the mice received a second Ehrlich tumor implant. **Methods:** For PDT, diode laser and Methylene Blue (MB) 1% as photosensitizer were used. Tumor growth was evaluated with a caliper for 17 days. At necropsy, spleen and lymph node were weighed; tumor samples were weighed and the H&E sections were analyzed through morphometry. Blood samples were taken for leukocyte count. **Results:** Tumor growth did not diverge between groups. Morphometric analysis of the second tumor showed that PDT group had a lower volume fraction of tumor cells and higher inflammatory infiltrate and necrosis area. The relative spleen weight was also higher in PDT group and white pulp hyperplasia was seen. Lymph node weight and blood counts were similar in both PDT and surgery groups. **Conclusion:** PDT with MB in primary Ehrlich tumor was able to induce inflammation and necrosis in a second implant of the same tumor, in comparison with surgically removed Ehrlich tumor. **Impact statement:** PDT can possibly induce an adaptive immune response to a secondary tumor, and as such may control initial steps of metastasis.



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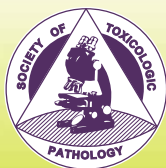
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Mass Spectrometry Imaging of Therapeutic Antibodies: Distribution of Unlabeled Trastuzumab in CB.17 SCID Mice Implanted with the Human Breast BT474 Xenograft

Category: New Technologies

David BONNEL¹, Chassidy HALL², Robert J MULLIN² Kathryn Simon² and Jonathan STAUBER¹
¹ImaBiotech, Lille, France, ²Charles River Discovery Research Services-, North Carolina –Morrisville, USA

The kidney serves several essential regulatory roles in the human body and the dysregulation of renal physiological properties can induce serious pathologies. Moreover, it could be a major site of organ damage caused by drug toxicity (i.e. nephrotoxicity). The kidney can be separated into three major parts; the cortex, the medulla and the pelvis with functional significance. These regions have several small subregions of a few micrometers scale, such as for example the renal corpuscles (glomeruli) or the tubules. There are different classes of lipids with specific role in the kidney cell proliferation, the cellular signaling or the inflammation process. For instance, dysfunction of sphingolipids (SL) and glycosphingolipids (GSL) metabolism induces the accumulation of these molecules in kidney substructures (e.g. glomeruli) which could result in different kidney diseases. Classical mass spectrometric analysis coupled with liquid chromatography (LC-MS) uses tissue homogenates, cannot provide spatial data. However, Mass Spectrometry Imaging (MSI) permits simultaneous detection and quantification of a wide range of molecules without labelling while keeping their spatial information at the low micrometer level. Some of the histological related lipids observed using MSI, such as gangliosides (glomeruli), cardiolipins (cortex) or sulfatides (medulla) are considered as reliable biomarkers of disease state. The detection of these markers within tissue section, in combination with the precise distribution of the drug candidate in these different kidney substructures provides large amount of information in support of PK/PD studies.



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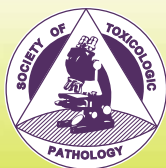
Mass Spectrometry Imaging in a Toxicology Study: Application in Induced Interstitial Pulmonary Fibrosis (IPF) Model

Category: New Technologies

David Bonnel¹, Mary McElroy², Emeline Falaux¹, Gael Picard De Muller¹, Fabien Pamelard¹, Stephen Madden², Jonathan Stauber¹

¹ImaBiotech, Lille, France, ²Charles River Discovery Research Services, Edinburgh, UK

The identification of the potential causes of the histopathologic/functional tissue changes is one of the main difficulties in toxicology studies. Mass spectrometry imaging (MSI) technology has been used to address these crucial issues. The main advantage of this label free imaging technique is the detection of all molecules of interest directly on-tissues with high specificity. Indeed the molecular distribution of some unlabeled targeted molecules could be directly correlated with some histopathologic and functional tissue changes. MSI was used to improve the understanding of the Bleomycin-induced interstitial pulmonary fibrosis rat model. Rats were administered seven doses of bleomycin delivered to the lungs and followed for 14 days. Control animals received seven doses of saline. Control and treated animals were sacrificed and lungs were collected. Several fresh sections were prepared and then molecular imaging was performed by high spectral resolution and high spatial resolution-MSI. Based on the combination of MSI and staining, lysophosphatidic acids (LPAs) were detected and confirmed to be specifically distributed in the fibrosis area. A significant difference of signal was observed for LPAs in the IPF tissues compared to the control tissues. LPA was described in the literature to contribute to the development of fibrosis after lung injury through multiple mechanisms (via LPA1 and LPA2 receptors). Moreover, some other specific ions of the fibrosis are currently being identified as well as some other potential markers at a higher mass range (>1000 Da). MSI provides the identification of markers/readouts in toxicology studies associated with atypical toxicologic pathology findings.



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Use of Legacy Data to Assess Species Concordance for Liver Injury

Category: New Technologies

Stephanie Berry¹, Paul Bradley¹

¹*Instem Scientific, Cambridge, UK*

During early drug development, a candidate is subjected to rigorous testing, the objective of this is to avoid progressing unsafe molecules through the development process. A key consideration for scientists is whether effects seen early on will lead to pathologies in humans.

Instem's Safety Intelligence Program (SIP) is a database describing the effects of compounds in different species and tissues. It is built from a variety of public data sources, such as Medline and the FDA NDAs. We will show how SIP can be used to assess the concordance of drug effects across different species.

Using SIP, we identified 830 active ingredients of drugs which affect clinical markers for liver injury (e.g. ALT, AST, bilirubin). We then delved further into 50 of these, to discover whether they led to liver injury in different species. Alongside this, we also looked at ~150 drugs that did not affect clinical markers for liver injury, and whether they went on to cause liver injury.

We were able to determine that 37 of the 50 compounds affecting liver injury markers caused liver damage in humans. 25 of those causing liver damage in humans, also caused it in rats, giving a 68% concordance. 18 of the drugs caused liver damage in dogs, but only 11 translated into humans, giving a 30% concordance.

The analysis demonstrates that legacy data from different sources can be used to determine drug effects from early testing through to market, and provide insight into the translational utility of non-human models.



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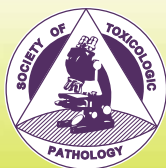
Pathology of IL-33 Knock-In Mouse Model of Non-Resolving Inflammation

Category: New Technologies

Maria Cristina De Vera Mudry¹, Juliana Bessa¹, Claas Aiko Meyer¹, Sonja Schlicht¹, Susan H. Smith², Thomas Weiser¹, Thomas Singer¹, Antonio Iglesias¹, Javier Cote-Sierra²

¹F. Hoffmann-La Roche Ltd., Basel, Switzerland, ²Stiefel, Research Triangle Park, NC, USA

Introduction: IL-33 is a pro-inflammatory cytokine that induces T_H2 responses (IL-5, eosinophilia). The wide distribution of IL-33 receptor (ST2) supports IL-33 involvement in diseases characterized by a non-resolving inflammation, such as asthma, arthritis, and enterocolitis. **Methods:** Male and female IL33^{tm1/+} (chromatin-binding domain replaced by dsRed) and IL33^{tm2/tm2} (cytokine domain replaced by dsRed) mice were generated on a BALB/c background. Mice were sacrificed at different ages. Tissues were fixed in 10% neutral buffered formalin, processed, stained (H&E, anti-IL-33/CD31), and examined by light microscopy. Serum IL-33 was measured by ELISA. **Results:** IL33^{tm1/+} mice appeared normal at birth, became morbid, and died from ~90 days old. Spleen and mesenteric lymph node enlargement correlated with significant hypercellularity (plasma cells, eosinophils). Thickening and reddening of the intestines correlated with villous thickening, mononuclear and eosinophilic infiltration, and hypertrophy/hyperplasia of smooth muscle cells. The lungs showed goblet cell hyperplasia, and eosinophilic and mononuclear inflammation. The femoro-tibial joint space was obliterated by synovial hyperplasia, and the articular surfaces were rough due to cartilage loss and inflammation. Other findings were inflammation of the heart, uterus and vagina, hepatic amyloidosis, and bone marrow eosinophilic myeloid hyperplasia. IL-33+ cells were increased in areas of inflammation. Hematology showed lymphocytosis and eosinophilia. Serum IL-33 was elevated. IL33^{tm2/tm2} and WT mice were normal. **Conclusion:** Lesions observed in IL33^{tm1/+} mice recapitulate the role of IL-33 in various non-resolving inflammatory conditions. **Impact statement:** The IL-33 knock-in mouse model of non-resolving inflammation is a tool for assessing the efficacy of anti-inflammatory compounds and biotherapeutics targeting IL-33.



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Integration of MALDI Imaging and Optical Microscopy Allows New Insights into Molecular Distribution in Tissue

Category: New Technologies

Katherine Kellersberger¹, M. Reid Groseclose², Stephen Castellino²

¹*Bruker Daltonics, Billerica, MA, USA*, ²*GlaxoSmithKline, Research Triangle Park, NC, USA*

Introduction: MALDI imaging mass spectrometry (IMS) is a technique that is successfully used to directly measure compounds such as drugs, metabolites, lipids, and proteins in tissue without antibodies or special staining. Because IMS is a non-targeted technique, multiple analytes can be evaluated in a single experiment then correlated to histology studies. In many cases, it is not until these two modalities are combined that a complete picture forms.

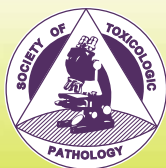
Experimental Design: Tissue sections from animals dosed with therapeutic drugs in DMPK studies were subjected to IMS analysis, while serial sections were subjected to traditional histological staining techniques. Molecular images were generated and co-registered with histology images to examine mechanisms of metabolite-induced toxicity in tissue.

Methods: Tissues were cryosectioned at 10 μ m, then coated with matrix solution (DHB, 30 mg/mL in 50% methanol) and subjected to IMS analysis using MALDI-FTMS. Serial sections underwent H&E staining. Molecular images were visualized using the FlexImaging software.

Results: Two case studies are presented; the first compares the distribution of a toxic metabolite in model animals to a metabolite found in patient CSF and proves affected animals had a drastically different distributions of the metabolite in brain than those unaffected. The second shows a link between metabolite distribution and inflammation.

Conclusions: Molecular histology workflows allow for detailed understanding of both histology and molecular information, revealing information on drug distribution that was previously impossible to obtain.

Impact Statement: High Definition MALDI imaging has shown utility in elucidating highly complex biological processes near single cell resolution in DMPK workflows.



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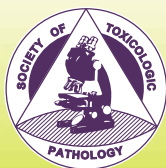
A SEND Solution for Microscopic Pathology, In-Life, and Other Preclinical Toxicology Data Collected Using Multiple LIMS (Laboratory Information Management Systems)

Category: New Technologies

Frederic Mura², Maro Schuster¹, Reto Aerni², Michael Wasko¹, Christina Wuermlin², Rich Buchanan¹, Amelia Bedoan², Laura Kaufman¹

¹Preclinical Data Systems, Mt. Arlington, NJ, USA, ²Preclinical Data Systems, Basel, Switzerland

Introduction: Toxicology studies in today's business environment involve multiple testing sites and LIMS. For example, it is not uncommon for microscopic pathology data to be collected on a different LIMS from that used for in-life. Moreover, as is done for "paper reports", all FDA-compliant SEND data sets need to make appropriate correlations, such as macroscopic and microscopic findings and, if appropriate, palpable masses and macroscopic findings. Microscopic data from carcinogenicity studies need to be mapped to SEND tumor.xpt files. Accordingly, SEND solutions need to harmonize potential heterogeneity of metadata, file formats, and terminology. **Materials and Methods:** We wish to report a web-based software architecture for SEND that accomplishes aggregation, harmonization and translation of data from different sources into one complete SEND dataset including all XPTs, define files and validation report(s). The architecture involves input from LIMS-specific or file based adapters, which are then processed through an engine that maps data to appropriate variables and domains; harmonizes data between different domains, such as animal and study number; consolidates comments (co) and relationships (relrec) such as macro/micro correlations; and performs controlled terminology translation. Trials information is provided to the engine through an application programming interface. **Results:** This SEND solution has been used successfully to submit SEND datasets created by multiple source systems to the FDA, including different LIMS for in-life and microscopic pathology. The poster presentation describes the software architecture in greater detail. **Conclusion and Impact:** It is possible and necessary to develop automated SEND solutions for preclinical studies involving multiple LIMS.



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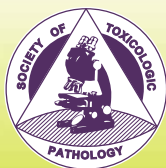
Characterizing and Diminishing Autofluorescence in Formalin-Fixed Paraffin-Embedded Human Respiratory Tissue

Category: New Technologies

A. Sally Davis^{1,2}, Anke Richter³, Steven Becker¹, Jenna Moyer¹, Aline Sandouk¹, Jeff Skinner¹, Jeffery Taubenberger¹

¹National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, ²North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA, ³Defense Resources Management Institute, School of International Studies, Naval Postgraduate School, Monterey, CA, USA

Introduction: Successful visualization of fluorescent markers in formalin-fixed paraffin-embedded (FFPE) respiratory tissue sections is frequently hampered by tissue autofluorescence, particularly in the green wavelengths. Because the vast majority of tissues for human and animal pathology over the last century has been FFPE, this study focused on reducing autofluorescence in typical FFPE sections. **Experimental design:** Focusing on FFPE human tracheal tissue layers of highest interest to respiratory pathologists and virologists, we first qualitatively assessed nine treatments reported to have efficacy in reducing autofluorescence in other tissue types. Second, we quantitatively compared the three most promising treatments and also considered the impact of antigen retrieval and serum blocking. **Methods:** Qualitative efficacy was scored on three dimensions: green wavelengths autofluorescence reduction, interference in other wavelengths, and treatment preparation and reproducibility. We characterized the autofluorescence signature by measuring tissue autofluorescence using the multi-lambda mapping technique on a Leica SP5 white light laser (WLL) confocal microscope and performing mathematical modeling. **Results:** The three most efficacious treatments were Eriochrome black T, Sudan black B and sodium borohydride. Functionally fitting this multi-lambda data to 2-dimensional Gaussian surfaces revealed that steam antigen retrieval and serum application contribute minimally to autofluorescence and that the three treatments are disparately efficacious. **Conclusion:** Together these studies provide a set of guidelines for diminishing autofluorescence in FFPE human respiratory tissue. **Impact Statement:** These techniques are transferable to similar questions in other tissue types and might also be used to quantify effects in studies where a treatment causes a change in tissue autofluorescence.



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The Use of Subcutaneously Implanted Transponders in the Tg.rasH2 Mouse

Category: Oncology/Carcinogenesis

Madhav Paranjpe¹, Jessica Belich¹, Reem Elbekai¹

¹BioReliance By SAFC, Rockville, MD, USA

Subcutaneous implantation of transponders is a commonly used method for animal identification in preclinical studies involving conventional rodents. However, no robust data is available to support the use of subcutaneous transponders in transgenic Tg.rasH2 mouse. The Tg.rasH2 mouse model is commonly used in 26-week carcinogenicity assessment studies, and the objective of this study is to determine if implantation of glass encapsulated microchip transponders increases the incidence of tumors in this model over a 26-week period. Male and female Tg.rasH2 mice were assigned to three groups. Group 1 consisted of mice in which the transponders were implanted subcutaneously. Group 2 consisted of mice in which transponders were not implanted but were subjected to subcutaneous injection procedure. The third group consisted of mice that were implanted with transponders but were also inoculated intra-peritoneally with positive control material, urethane. In none of the mice there was tumor formation at the site of injection. The most common finding was a cavity formation at the transponder site with surrounding compressed connective tissue. In few mice, there was infiltration of inflammatory cells around these cavities. The incidence of spontaneous tumors in these mice remained within the historical control range. As expected, the positive control material urethane induced pulmonary tumors and splenic hemangiosarcomas. It is therefore concluded that the use of transponders Tg.rasH2 mice is safe and would not negatively impact the outcome of a 26-week carcinogenicity study.



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Evaluation of Key Events in the Mode of Action for a Carry-Over Carcinogen in Mice

Category: Oncology/Carcinogenesis

Charles Wood¹, April Lake^{1,2}, Greg Olson³, Michael George¹, Susan Hester¹, Anthony DeAngelo¹

¹US Environmental Protection Agency, Research Triangle Park, NC, USA, ²University of North Carolina-Chapel Hill, Chapel Hill, NC, USA, ³Toxicologic Pathology Associates, Jefferson, AK, USA

Introduction: Early life environmental exposures are established determinants for adverse health outcomes later in life, although epigenetic drivers for these effects are not currently known. Previously we found that postnatal exposure to dichloroacetic acid (DCA), a common drinking water disinfection byproduct, increased liver tumorigenesis in mice 80 weeks after exposure was stopped. Here we evaluated time course dynamics of key events related to this effect. **Experimental Design:** The study followed a stop-promotion design in which 28-day old male B6C3F1 mice were given the following treatments in drinking water for up to 93 weeks: deionized water (dH₂O; control); 3.5 g/l DCA continuously; or 3.5 g/l DCA for 4, 10, 26, or 52 weeks followed by control dH₂O. Endpoints included liver tumorigenesis, cytotoxicity, and quantitative cell proliferation evaluated across eight time points. **Results:** Liver tumor incidence was increased in all DCA treatment groups. No group differences in preneoplastic foci were observed. Minimal hepatocellular necrosis was observed with direct DCA exposure, but this effect did not persist after stopping DCA. Prior DCA treatment did not result in increased liver cell proliferation.

Conclusion: Transient early adult DCA treatment of relatively short duration (4 weeks) captured the majority of carcinogenic effects resulting from lifetime exposure. This carry-over effect was not associated with sustained cytotoxicity, increased cell proliferation, or preneoplastic lesions. **Impact Statement:** Key intermediate events resulting from early life DCA exposure do not fit the classical mitogenic or cytotoxic modes of action for non-genotoxic carcinogenesis. Alternative epigenetic mechanisms for this distinctive effect are currently unidentified.



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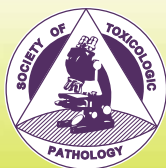
Immunohistochemical Characterization of Carbon Nanotube-Induced Malignant Mesotheliomas in Rats

Category: Oncology/Carcinogenesis

Susanne Rittinghausen¹, Anja Hackbarth¹, Heinrich Ernst¹, Uwe Heinrich¹, Albrecht Leonhardt², Dirk Schaudien¹

¹Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany, ²Leibniz Institute for Solid State and Materials Research, Dresden, Germany

Introduction: Biological effects of tailor-made multi-walled carbon nanotubes (MWCNTs) were investigated in vivo in a 2-year carcinogenicity study in a project funded by the German Federal Ministry of Education and Research (contract no. 03X0109A). In the past, intraperitoneal (i.p.) carcinogenicity studies in rats using biopersistent granular dusts with very high mass doses were always negative, whereas a number of such i.p. studies with different asbestos fibers showed tumor induction. **Methods and Materials:** Fifty Wistar rats per group were treated once by i.p. injection of a low (1x10⁹ WHO fibers) or high (5x10⁹ WHO fibers) dose of different MWCNTs (MWCNT1, 2, 3, and 3a) suspended in artificial lung medium that was also used as negative control. Amosite asbestos (0.1x10⁹ WHO-fibers) served as positive control. Moribund rats were sacrificed and necropsy comprising all organs was performed. Histopathological classification of tumors and immunohistochemistry for podoplanin, Wilm's tumor antigen 1, cytokeratin, vimentin, desmin, smooth muscle actin, ICAM-1, MCAM, thrombomodulin, MMP-14, CD90, CD147 and Ki-67 were conducted to compare the induced tumors with mesotheliomas occurring in humans. **Results:** The treatments induced mesotheliomas in all dose groups, whereas incidence and time to tumor were different between the groups. **Conclusion:** Tumors induced by i.p. injection of different MWCNTs and of asbestos were histopathologically and immunohistochemically similar, also in comparison to mesotheliomas in men, suggesting an analog pathogenesis. **Impact Statement:** Based on our results of the carcinogenicity study with different MWCNTs, the accreditation and production of MWCNT-fibers need further regulations.



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Proliferative Lesions in the Rete Ovarii of Aged Han Wistar Rats

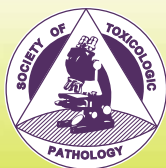
Category: Oncology/Carcinogenesis

Richard Haworth¹, Jaimini Kumar¹, David Lewis¹

¹GlaxoSmithKline, Ware, UK

Introduction: The rete ovarii (rete) is the homologue of the rete testis and reported lesions of the rete are rare in the rat. **Experimental Design:** Ovaries from control Han Wistar rats were examined from two carcinogenicity studies. In study 1, there were 120 control rats with a terminal euthanasia at 104 weeks. In study 2, there were 280 control rats with interim euthanasia at 12, 18 months and the terminal euthanasia at 28 months. **Methods:** Both ovaries were conventionally fixed and stained with haematoxylin and eosin. Selected sections were also immunostained by α -inhibin and pan-cytokeratin. **Results:** In study 1, the only proliferative lesion observed was rete hyperplasia in 19/120 rats (15.8%). In study 2, the incidence of rete hyperplasia was similar (48/280: 17.1%) and increased with age. In study 2, there was also a low incidence of rete ovarii adenoma (4/250; 1.4%). Intra-tubular rete ovarii adenomas were characterised by distension of the affected rete tubule by an intratubular mass of proliferative cells. There was minimal α -inhibin positive cells in normal or cystic rete epithelia, but they became increasingly more prominent in the higher grades of hyperplasia and in adenomas. **Conclusion and Impact statement:** This is the first description of the microscopic appearance and incidence of proliferative ovarian rete lesions in rats and will enable more widespread recording of this change.

"All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals."



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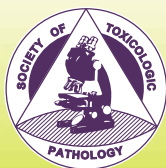
Early Detection of Carcinogenic Potential of CNT via Cell Proliferation Measurement

Category: Oncology/Carcinogenesis

Dirk Schaudien¹, Anja Hackbarth¹, Heinrich Ernst¹, Albrecht Leonhardt², Uwe Heinrich¹, Susanne Rittinghausen¹

¹Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany, ²Leibniz Institute for Solid State and Materials Research, Dresden, Germany

Introduction: Multiwall carbon nanotubes (MWCNT) are discussed to have a toxic potency depending on their length and fiber-like shape. To investigate potential early recognition of carcinogenic behavior of MWCNT, they were injected intraperitoneally in rats and the cell proliferation rate was measured at the diaphragmatic peritoneum in a project funded by the German BMBF. **Methods:** Tailor-made MWCNT (1, 2, 3) with different lengths and diameters were produced, suspended in artificial lung medium and injected intraperitoneally in rats in two dose groups (low: 1×10^9 WHO-fibers; high: 5×10^9 WHO-fibers). Long amosite asbestos (0.1×10^9 WHO-fibers) served as positive, ground MWCNT and Printex 90 (5 mg/rat) as particle negative controls. Three, 6 and 12 months after injection of fibers, the animals were necropsied, the thickness of the peritoneum was measured, and the cell proliferation rate was determined using the BrdU method. The acquired data were correlated with a parallel carcinogenicity study. **Results:** There was a time-independent significant increase in the cell proliferation rate of MWCNT 1_(high) (length=7.9 μ m; diameter=0.037 μ m), MWCNT 2_(low/high) (length=10.24 μ m; diameter=0.04 μ m) and MWCNT 3_(low/high) (length=8.57 μ m; diameter=0.085 μ m) comparable to amosite asbestos (length=13.95 μ m; diameter=0.39 μ m). In the parallel carcinogenicity study, MWCNT 2 and 3 showed a higher mesothelioma incidence than MWCNT 1. **Conclusion:** Some MWCNT mediate enhanced proliferation of peritoneal cells of the diaphragm in rats which correlated well with results of the parallel carcinogenicity study. **Impact Statement:** Early detection of carcinogenic potential of CNT via cell proliferation measurement of the diaphragmatic peritoneum after intraperitoneal injection in rats is possible.



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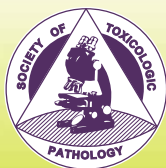
The RITA Database—The Value of Incidences of Tumors in Young Animals

Category: Oncology/Carcinogenesis

Dirk Schaudien^{1,3}, Rupert Kellner^{1,3}, Matthias Rinke^{2,3}

¹Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany, ²Bayer Pharma AG, Wuppertal, Germany, ³RITA-Registry of Industrial Toxicology Animal-Data, Hannover, Germany

Introduction: Tumors in young animals are considered as a relatively rare event. However, their presence might evoke concerns especially when they do occur in treated animals in short-term studies. There are a few publications available that address this topic. **Methods:** The RITA database contains historical control data of peer-reviewed histopathological diagnoses of tumors and other proliferative lesions. The database reflects data from more than 26,000 rodents of carcinogenicity studies. Animals which died or were euthanized moribundly at an age younger than 6, between 6 and 12 months or older than 12 months were selected from the database. Tumor incidences of these three groups were compared to each other. **Results:** The earliest tumor occurring was an adenoma of the distal part of the pituitary gland in a 61 days old female Wistar rat. The tumor incidence of rats younger than 6 months was 18%, at an age between 6 and 12 months 44%, and in rats older than 12 months 80%. For mice the incidences were 12% in animals younger than 6 months, 23% for animals between 6 and 12 months of age, and 60% for animals older than 12 months. **Conclusion:** Since tumors do occasionally occur already in young animals historical control data is also needed for these incidences. Therefore, the RITA group is planning to include tumor and pre-neoplastic incidences from studies with shorter duration (e.g. 13 or 26 weeks) in the future. **Impact statement:** Further knowledge about tumor incidences of young animals will improve evaluation of short-term studies.



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Effect of Oral (Gavage) Administration of *Euphorbia tirucalli* (Aveloz) on the Ascitic Ehrlich Tumor Growth

Category: Oncology/Carcinogenesis

Diego Pinha Alvez da Cruz¹, Gregory Menecier¹, Andréia Latorre², Gokithi Akisue³, Mitsue Haraguchi⁴, Roueda Abu-Said⁵, Maria Lucia Zaidan Dagli¹

¹University of São Paulo, São Paulo, Brazil, ²Adolpho Lutz Institute, São Paulo, Brazil, ³University of São Francisco, Bragança Paulista, Brazil, ⁴Biological Institute, São Paulo, Brazil, ⁵University of Santa Cruz, Ilhéus, Brazil

Introduction: *Euphorbia tirucalli* presents several bioactive components and antineoplastic activities have been reported in folk medicine in Brazil. **Objectives:** The effects of *E. tirucalli* was studied in mice bearing ascitic Ehrlich tumor. *E. tirucalli* was planted in the University of São Paulo, and its latex was used throughout the experiments. **Methods and materials:** Four groups of mice received, by gavage, respectively 0, 0,0123%, 0,0246% or 0,0369% of *E. tirucalli* latex in saline solution for 14 days. At the end of the period, complete necropsy and histopathology were performed. In a second study, two groups of 15 male C57Bl/6 mice received i.p 9 x 10⁶ Ehrlich tumor cells, and the mice received either 0 % or 0,0369% of *E. tirucalli* latex diluted in physiological saline solution, per gavage, for 14 days. At the end of the period, ascitic tumor cells were counted, and separated as viable or non viable by the Trypan blue dye exclusion method. **Results:** No signs of toxicity of *E. tirucalli* latex were observed with the given doses. A significant reduction in the number of Ehrlich ascitic tumor cells was observed in the treated mouse group (90,83 ± 22,68 versus 128,12 ± 28,85 cells). In addition, a significant increase in the number of non viable cells was also observed in the treated group (34,23 ± 13,59 versus 13,88 ± 4,83 cells in the control group). In conclusion, *Euphorbia tirucalli* latex presents antineoplastic effects on this transplantable tumor. **Impact statement:** *E. tirucalli* latex deserves further investigation as a promising antineoplastic plant product.



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Immunohistochemical Characterization of ENU-Induced Brain Tumors in F344 Rats

Category: Oncology/Carcinogenesis

Rebecca Moore¹, Holly Kolenda-Roberts², Nancy Harris¹, Young-Man Cho³, Kumiko Ogawa³, Jerry Hardisty¹, Rodney Miller¹

¹Experimental Pathology Laboratories, Inc., Research Triangle Park, NC, USA, ²SNBL USA, Ltd., Everett, WA, USA, ³National Institute of Health Sciences, Tokyo, Japan

Introduction: N-ethyl-N-nitrosourea (ENU) is an alkylating agent that is used extensively in experimental neuro-oncogenesis. In rats, ENU causes neural neoplasms in up to 100% of offspring of dams given a single dose in the second half of gestation. **Experimental Design:** Immunohistochemical staining was performed to further characterize 26 ENU-induced brain tumors (8 glioblastomas, 11 astrocytomas, and 7 oligodendrogliomas) from a study in which 20 pregnant Fischer 344 rats were given 20 mg/kg ENU intravenously 4 days before giving birth. **Materials and Methods:** Hematoxylin & eosin slides were prepared from each animal. Immunohistochemical stains included glial fibrillary acidic protein (GFAP), ionized calcium-binding adapter molecule 1 (Iba-1), and oligodendrocyte transcription factor 2 (Olig2). **Results:** A total of 19 tumors were observed and diagnosed as oligodendrogliomas. Neoplastic cells stained most consistently with Olig2. There were 5 well-differentiated oligodendrogliomas. The remaining 14 tumors exhibited considerable cellular anaplasia and corresponding variability in Olig2 staining. These tumors also contained greater numbers of Iba-1+ microglial cells (diffuse) and GFAP+ astrocytic cells (largely distributed at the tumor edge and around blood vessels). The "ependymoma-like," pseudorosette features of ENU-induced tumors stained positively for Iba-1, suggesting reactive microglia/macrophages. **Conclusion:** ENU-induced neoplasms consisted of variably differentiated oligodendrogliomas. While some tumors were well-differentiated, others were anaplastic with robust secondary infiltrates of reactive cells (microglial, astrocytic, endothelial). **Impact Statement:** A previous study indicated most spontaneous tumors in the rat were oligodendrogliomas, while chemically-induced neoplasms were malignant microglial tumors. Results for ENU are unique thus far for its induction of variably differentiated oligodendrogliomas.



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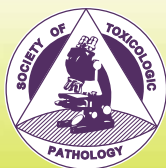
Influence of Processing Methods for Scanning Electron Microscopy in the Detection of Diuron-Induced Ultrastructural Alterations in the Rat Urinary Bladder

Category: Oncology/Carcinogenesis

Rafaela Fava¹, Ana Paula Cardoso¹, Mitscheli da Rocha¹, Merielen Nascimento e Pontes¹, João Lauro de Camargo¹, Maria Luiza de Oliveira¹

¹UNESP-São Paulo State University, Botucatu, Brazil

Introduction: Cytotoxicity and necrosis followed by regenerative hyperplasia is the proposed carcinogenic mode of action (MoA) of the herbicide diuron in the rat urinary bladder. Early morphological alterations putatively related to cytotoxicity are preneoplastic swollen cells as observed under scanning electron microscopy (SEM). However, these cells have not been observed in previous studies conducted at our laboratory after SEM processing methods different from those used when they were described. The present study aimed to evaluate the influence of SEM processing methods on the detection of urothelial changes induced by diuron, particularly the swollen cells. **Experimental Design:** Male Wistar rats divided in control, sodium saccharin 7.1% (NaS, positive control) and diuron 2500ppm groups, and fed for 7-days or 15-weeks. **Methods:** Urinary bladders fixed with Bouin or glutaraldehyde were processed for SEM using critical point drying (CPD) or hexamethyldisilazane (HMDS). Histological and cell proliferation evaluations were also performed. **Results:** After seven days, differences of histological lesions incidences or labeling indices were not detected; however, after 15 weeks the incidence of simple hyperplasia was increased in the animals fed NaS and diuron. Under SEM, the incidences of urothelial alterations were higher in diuron-fed animals at both moments, but swollen cells were seen only at the seven-day study regardless the processing method used. **Conclusion:** Our results confirm that preneoplastic swollen cells are early event during the diuron-induced urothelial carcinogenesis process and not a processing artifact. **Impact statement:** SEM can be useful for detecting early cytotoxicity in the urinary bladder.



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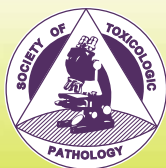
Role of Histone Citrullination in Macrophage Extracellular Trap (MET) Release and Characterization of METs in Human Tongue Squamous Cell Carcinoma

Category: Oncology/Carcinogenesis

Sunish Mohanan^{1,2}, Angela Yan², Lynne Anguish², Dalton McLean², Sachi Horibata², Paul Thompson⁴, Neil A. Iyengar³, Andrew J. Dannenberg³, Scott A. Coonrod²

¹Eli Lilly and Company, Indianapolis, IN, USA, ²Baker Institute, Cornell University, Ithaca, NY, USA, ³Weill School of Medicine, Cornell University, New York, NY, USA, ⁴The Scripps Research Institute, Jupiter, FL, USA

Peptidylarginine deiminase (PAD)-mediated histone citrullination has been found to play a critical role in the release of chromatin-containing extracellular traps ("ETosis") from immune cells. We have previously demonstrated that site-specific histone citrullination marks such as Histone H4 Citrulline 3 (H4Cit3) are abundantly represented in neutrophil extracellular traps (NETs). Following the observation that macrophages express high levels of the PAD isozyme, PAD2, we hypothesized that PAD2 may promote the formation macrophage extracellular trap (METs). Using RAW264.7 and primary peritoneal macrophages, we showed that PAD2 is involved in ET release and that PAD2 deletion and drug-mediated PAD inhibition in macrophages affect the ability of macrophages to release extracellular chromatin traps following chemical stimulation by phorbol myristate acetate (PMA). Additionally, the morphological characteristics of METs were affected in the absence of nuclear PAD2. Macrophages represent a major inflammatory component within the microenvironment of various carcinomas and sarcomas. Therefore, to test whether METs may form within a clinically relevant setting, we have also characterized the presence of METs in human tongue squamous cell carcinomas. Immunostaining and confocal analysis of these sections found that tumor associated macrophages (TAMs) express high levels of nuclear and cytoplasmic PAD2 and that clusters of the TAMs appear to form METs, as evidenced by the presence of extracellular DNA and citrullinated histones. Our study supports the hypothesis that PAD2 is involved in MET release and that PAD2-mediated MET release may play a role in regulating the inflammatory microenvironment in human carcinomas.



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NNK and Enantiomers of Its Metabolite, NNAL, Constituents of Tobacco Products, Induce Lung Tumors That Metastasize to Pancreas in F-344 Rats

Category: Oncology/Carcinogenesis

Ramesh C. Kovi^{1,2}, Charles S. Johnson^{1,2}, Silvia Balbo², Stephen S. Hecht², M. Gerard O'Sullivan^{1,2}

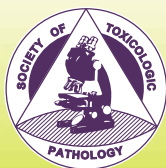
¹*Comparative Pathology Shared Resource, St. Paul, MN, USA,* ²*Masoni Cancer Center, University of Minnesota, Twin Cities Campus, MN, USA*

Introduction: The tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), is metabolized to enantiomers of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which are found in the urine of virtually all people exposed to tobacco products. NNK and racemic NNAL are known to induce lung and pancreatic tumors in rats, but the carcinogenicity of NNAL's enantiomers has not been studied previously.

Methods: Male F-344 rats were dosed with (R)-NNAL (5ppm, n=24), (S)-NNAL (5ppm, n=22), racemic NNAL (10ppm, n=15), NNK (5ppm, n=24) or water (control, n=22) for up to 90 weeks prior to necropsy.

Results: All test compounds induced a high incidence of lung tumors. NNK and racemic NNAL were the most potent; (R)-NNAL and (S)-NNAL had equivalent activity. The spectrum of treatment-related histopathological findings comprised pulmonary bronchoalveolar epithelial hyperplasia, adenomas, and carcinomas, and pancreatic tumors. Pulmonary carcinomas frequently exhibited local invasion/metastasis within the mediastinum and thoracic cavity. Immunohistochemical characterization of the lung and pancreatic tumors using prosurfactant protein C, CC-10 and CK-19 markers indicated that the pancreatic tumors are metastatic tumors from lung.

Discussion: This study demonstrates that the enantiomers of NNAL are potent pulmonary carcinogens in rats, and confirm previous findings regarding the carcinogenicity of NNK and racemic NNAL. It further confirmed the aggressive nature of induced pulmonary carcinomas, which is reflected in local invasion/metastasis within the thoracic cavity, and distant metastasis to the pancreas. Our observation regarding metastasis to the pancreas was an important, and unexpected, finding in this study. These findings underline the urgent need for immediate regulation of human exposure to NNK and NNAL.



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Hitherto Unknown Type of Adenocarcinoma in the Rat Mammary Gland

Category: Oncology/Carcinogenesis

Heike Marxfeld¹, Silke Treumann¹, Karin Küttler¹, Sibylle Gröters¹, Bennard van Ravenzwaay¹

¹BASF SE, Ludwigshafen, Germany

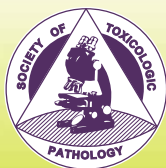
Introduction: The morphology and hormone receptor pattern of an unknown type of adenocarcinoma of the rat was investigated. The female rat was a control animal in a carcinogenicity study.

Experimental design: A control carcinogenicity study was performed with HanBrl:WIST (SPF) rats to generate historical control data for this strain.

Materials and Methods: Tumor tissue of a female control rat from a carcinogenicity study which died spontaneously after 728 days on study was investigated. Macroscopically, a 15 mm diameter cyst was noted in the right inguinal side of the mammary gland. Hematoxylin-Eosin stained slides and serial sections stained immunohistochemically with antibodies against estrogen receptor, progesterone receptor and androgen receptor were evaluated.

Results: A mammary tumor with features of sebaceous gland differentiation is described with the expression pattern of nuclear hormone receptors in tumor cells. "Mammary adenocarcinoma with sebaceous differentiation" is the proposed diagnosis.

Impact statement: A new diagnosis is proposed to be included in the INHAND nomenclature.



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Epigenetic Regulation of Transcription Factor Promoter Regions by Low-Dose Genistein through MAPK and MSK1 Nongenomic Signaling

Category: Oncology/Carcinogenesis

Linda Yu¹, Kyle Ham¹, Xiaohua Gao¹, Lysandra Castro¹, Norris Flagler¹, Ray Dong¹, Grace E. Kissling², Trevor K. Archer², Darlene Dixon¹

¹NIEHS, NTP, Research Triangle Park, NC, USA, ²NIEHS, Research Triangle Park, NC, USA

Introduction: Genistein binds to ERa and b, mimicking the effects of estrogen, when ERa is in excess. Genistein at low doses is involved in nongenomic activation of MAPK_{p44/42} via ERa leading to proliferation of human uterine leiomyoma cells. **Experimental Design:** Immortalized human uterine leiomyoma (ht-UtLM) and uterine smooth muscle cells (ht-UtSMCs) were used to evaluate if 1mg/ml of genistein could epigenetically modify histone H3 by phosphorylation at serine 10 (H3S10ph), and if so, is phosphorylation mediated by MSK1, downstream of MAPK_{p44/42}. **Methods:** Western blotting was used to assess MAPK_{p44/42}, MSK1, and H3S10ph expression and activation, with or without MAPK inhibitor (PD) in cells. Immunohistochemical studies were done to determine expression of MSK1 and H3S10ph in human leiomyoma and normal myometrial tissues. RT-PCR arrays were done to detect transcription factor genes. ChIP assays were used to confirm enrichment of the transcriptional factor promoter regions of genes found upregulated in the RT-PCR arrays. **Results:** In ht-UtLM cells, genistein activated MAPK_{p44/42}, MSK1, and H3S10ph, and PD inhibited these effects. Similar results were observed in human uterine leiomyoma tissue; however, none of these effects were observed in ht-UtSMCs or myometrial tissue. Transcriptional factor genes, EGR1, Elk1, ID1, and cMyb, were upregulated, and their promoter regions were enriched through genistein stimulation, which was inhibited by PD. **Conclusion:** Genistein epigenetically modified histone H3 by phosphorylation of serine 10, which was regulated by MSK1 and MAPK activation. **Impact statement:** Histone H3 phosphorylation possibly represents a mechanism whereby cell proliferation occurs following low-dose genistein exposure.



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Inhibitory Effects of Pequi (*Caryocar brasiliense* Camb) Oil on Preneoplastic Lesions in a Mouse Hepatocarcinogenesis Model

Category: Oncology/Carcinogenesis

Simone Morais Palmeira¹, Paula Regina Pereira Silva¹, Cesar Kopi Grisoli², Paulo Hilário Nascimento Saldiva¹, Juliana Shimara Pires Ferrão¹, Maria Lucia Zaidan Dagli¹, Francisco Javier Hernandez-Blazquez¹

¹University of São Paulo, São Paulo, Brazil, ²Federal University of Brasília, Brasília, Brazil

Introduction: Medicinal plants and the research of active substances in plants is a field that has been extensively investigated recently for protective effects on human and animal carcinogenesis. The pequi (*Caryocar brasiliense* Camb.) fruit, a native plant from the central region of Brazil contains several antioxidant substances in its oil extract, which are reportedly involved with the reduction of the risk of degenerative diseases. Here we evaluated the chemopreventive potential of pequi oil against preneoplastic hepatic lesions chemically induced by diethylnitrosamine in the infant mouse model. **Methods:** Diethylnitrosamine (DEN) was intraperitoneally injected in 15 day-old mice (10µg/g) as initiator. Five experimental groups were formed DEN (positive control, DEN); DEN100 (DEN + pequi oil by daily gavage 100 mg from the 30th day to the 189th day); DEN400 (DEN + pequi oil by daily gavage 400 mg, same protocol); PO400 (control, pequi oil 400mg) and C (negative control). **Results:** The diethylnitrosamine induced preneoplastic lesions (acidophilic hepatocyte foci, AHF) in 100% of animals in DEN groups and DEN100, but only 80% of the animals from DEN400 showed AHF and none was observed in the PO400 group, indicating a possible pequi oil protection against the development of the AHF. **Conclusion:** Further evaluation of the efficacy of the pequi oil in the protection against the development of preneoplastic lesions will be carried out by stereological and immunohistochemical analysis of cytokeratins 8/18 (CK 8/18) and CK 19 expression. **Impact Statement:** Positive results will indicate this oil as a chemopreventive agent for man and animals.



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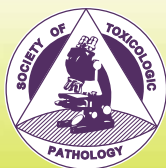
Multifunctional Photo-Theranostic Cancer Targeting Nanoporphyrin

Category: Oncology/Carcinogenesis

Tzu-yin Lin¹, Yuanpei Li¹, Luo Yan¹, Qiangqiang Liu¹, Sebastian Wachsmann-Hogiu¹, Chongxian Pan¹, Kit Lam¹

¹University of California-Davis, Sacramento, CA, USA

Introduction: Nanomedicine has great potential for cancer management. **Experimental design:** Newly developed porphyrin-based nanomicelles, nanoporphyrin (NP), was studied for the tumor selective delivery properties in imaging diagnosis (MRI, PET, NIR), photodynamic therapy, thermal therapy, and combined therapy (Chemo-PDT/PTT). **Methods:** Cancer cell lines were treated with NP and Doxorubicin-loaded NP (NP-DOX) followed by light exposure; cell viability, mitochondria potentials, apoptosis marker, and ROS production were measured. Mice bearing xenograft ovarian tumor or transgenic mice with breast cancers were treated with PBS, NP, and NP-DOX followed by light illumination, tumor size, temperature, ROS, apoptosis were measured. Mice with xenograft tumors were treated with NP, gadolinium (Gd) or ⁶⁴Cu loaded NP for NIR, MRI, PET imaging. **Results:** NP and NP-DOX mediated PDT caused concentration and light dose-dependent cytotoxicity with evidence of decreased mitochondria potentials, increased caspase 3/7 activities, apoptosis, DNA degradation (sub-G1), and ROS production. NP-DOX-PDT/PTT treated cancer-bearing mice/transgenic mice showed significant tumor suppression compared to PBS, NP-PDT/PTT, and NP-DOX treated groups. NP-PDT/PTT treated tumors were found to have increased temperature, ROS production, caspase 3 expression, and DNA damage. Intravesical administration of NP into orthotopic bladder cancer mouse model showed high tumor uptake signals compared to background urothelial cells. Finally, mice with intravenous injection of NP, NP-Gd, or NP-⁶⁴Cu exhibited obvious tumor signal accumulation on NIR, MRI and PET imaging and were used for follow-up. **Conclusion & Impact Statement:** This novel photo-theranostic nanoporphyrin not only integrated multiple clinical relevant functions with specific cancer targeting, programmable releasing, triggerable multiphase properties, but also could be a powerful “all-in-one” platform for personalized medicine.



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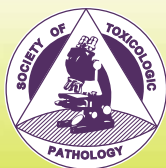
Post-Natal Development of the Ovary in the Rat

Category: Systemic/Organ-Specific Toxicologic Pathology

Catherine Picut¹, Amara Remick¹, Lydia Parker¹, Cynthia Swanson¹, Darlene Dixon²

¹WIL Research, LLC, Hillsborough, NC, USA, ²NIEHS, Research Triangle Park, NC, USA

Introduction: This study documents stage-specific microscopic features of the pre-pubertal ovary in the rat from postnatal day (PND) 3 through the peri-pubertal period (PND 36). **Experimental Design/Materials and Methods:** Ovaries from Sprague Dawley rats (3 animals per day) were collected from PND 3 through PND 36, fixed in formalin, sectioned at 5 μ m and stained with H&E. **Results:** During the neonatal period (PND 3 – PND 7), primordial follicles were the predominant feature with densely packed 1° and 2° follicles in the core. During the early infantile period (PND 8-14), the predominant feature was “atypical” 2° follicles, often arranged in linear arrays reminiscent of ovigerous nests. The late infantile period (PND 15-20) was characterized by a predominance of “mature” 2° and early 3° follicles, which were evenly distributed throughout the parenchyma. Waves of follicular atresia depleting the medullary follicles characterized the juvenile period (PND 21-32), and Graafian follicles (0.9-1 mm in diameter) characterized the peri-pubertal period (PND 33-36). The microscopic features of the neonatal, infantile, juvenile and peri-pubertal ovary are correlated with published changes in luteinizing hormone (LH)/follicle stimulating hormone (FSH) levels, changes in the excitatory or inhibitory nature of γ -aminobutyric acid (GABA), development of the estrogen positive feedback loop, and adrenarche. **Conclusion/Impact Statement:** Knowing the stage-specific features of ovarian development and their correlation to the neuroendocrine profile allows the pathologist to identify time-sensitive stages, and to better interpret morphologic changes in the ovary for the pubertal assay (PND 43) and juvenile toxicity studies.



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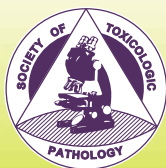
Post-Natal Development of the Testes in the Rat

Category: Systemic/Organ-Specific Toxicologic Pathology

Catherine Picut¹, Cynthia Swanson¹, Lydia Parker¹, Amera Remick¹

¹WIL Research, LLC, Hillsborough, NC, USA

Introduction: This study documents stage-specific microscopic features of the developing testis in the rat from birth through puberty (PND 55). **Experimental Design/Materials and Methods:** Testes from 159 Sprague-Dawley rats (3 animals per day from PND 3 through PND 55) were fixed in modified Davidson's fixative, sectioned at 5 μ m, stained with H&E and PAS-H, and examined by light microscopy. **Results:** During the neonatal period (PND 3–7), tubules were comprised of gonocytes and contained characteristic mitotically-active immature Sertoli cells. During the early infantile period (PND 8–14), profound proliferation of spermatogonia into a pseudostratified layer occurred, while Sertoli cells continued to proliferate as their nuclei retreated to a basilar location. During the late infantile period (PND 15–20) the crowded spermatogonia reached maximum density forming a characteristic rosette. Leptotene/zygotene spermatocytes appeared centrally as a tubule lumen developed, and inter-tubular staging became apparent. The juvenile period (PND 21–32) was characterized by a dramatic increase in number and size of pachytene spermatocytes with formation of round spermatids at PND 26, and loss of "infantile" rosette architecture. The hallmark feature of the peri-pubertal period (PND 32–52) was spermiogenesis with stage VII tubules visible at PND 46. **Conclusion/Impact Statement:** Knowing stage-specific features of testes during postnatal development help the pathologist interpret pubertal assays and juvenile toxicity studies; to distinguish delayed development from other toxic effects; and determine pathogenesis when confronted with non-specific findings of hypospermatogenesis.



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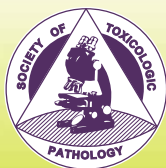
The Estrous Cycle in Young Sexually Mature Göttingen Minipigs: A Morphologic Approach

Category: Systemic/Organ-Specific Toxicologic Pathology

Eveline de Rijk¹, Birgit Peter¹, Joost Lensen¹, Helle Lorentsen², Hetty van den Brink¹

¹WIL Research Europe B.V., 's-Hertogenbosch, The Netherlands, ²Ellegaard Göttingen Minipig A/S, Dalmose, Denmark

Introduction: Minipigs are increasingly used in preclinical studies. Females are frequently young, increasing the chance on sexual immaturity/peripubertal and hampering a reliable interpretation of reproductive functioning. Moreover, a detailed description of the normal morphology during the estrous cycle is lacking. In the present study a marker to determine sexual maturity is provided and cycle phase-specific morphologic indicators are given enabling staging. **Experimental Design:** Twelve Göttingen female minipigs were scheduled for necropsy after the first progesterone peak at different cyclic phases, based on expected subsequent peaks. **Methods:** Blood samples for progesterone determination were taken twice weekly. After necropsy (age 5-8 months), ovaries, uterus, cervix, vagina and mammary gland were processed for histomorphologic evaluation (including HE- and/or PAS-staining). **Results:** Progesterone peaks correlated with the presence of corpora lutea (CL's), demonstrating ovulation. When necropsy occurred at Peak 1, fresh CL's were present, at Peak 2 fresh and degenerating CL's and at Peak 3 fresh, degenerating CL's and corpora albicans were found. Stage-distinguishing features comprise ovarian follicles and CL's (size/structure) and uterine epithelial morphology (cellular size, secretory activity, mitosis/apoptosis). Other organs examined were not suitable for staging due to high morphologic variability or absence of differences between phases. **Conclusion:** A progesterone peak is a good indicator for sexual maturity in female minipigs. Morphologic evaluation of ovary and uterus sections enables cyclic staging. **Impact statement:** Based on our study, an indicator for sexual maturity and a guidance for estrous cycle staging is provided, facilitating the evaluation of female minipig reproductive function within toxicity studies.



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Diurnal Variation in Clinical Chemistry Indicators of Liver Injury in Rats and Dogs

Category: Systemic/Organ-Specific Toxicologic Pathology

Mehrdad Ameri¹, Ryan Morgan¹, Kim Stocking¹, Marnie Higgins-Garn¹

¹*Comparative Biology and Safety Sciences, Amgen Inc., Thousand Oaks, CA, USA*

Introduction: Diurnal variation in clinical chemistry indicators of liver injury has been shown in human; however, limited data is available for preclinical animals. The purpose of this study was to assess alterations in selected clinical chemistry indicators of liver injury in naïve rats and dogs over a 24 hour time course. **Experimental Design:** Twenty Sprague Dawley rats (males and females, about 10 weeks old) and 10 beagle dogs (males, 13-14 months) were included in this study. **Materials and Methods:** Blood was collected at 5, 15, 30, 45, 60, 120, 360, or 1440 minutes post study start. For rats, blood was collected from a jugular cannula for the first 7 time points and via cardiac puncture at termination. For dogs, blood was collected by venipuncture from a cephalic or jugular vein. The following parameters were determined: ALT, AST, GLDH, ALP, GGT, total bilirubin, and total bile acids. **Results:** Mean and individual values were consistent across time points and in rats between males and females and intra-animal variability was generally low (<20% difference between initial and subsequent measurements). Although intra-animal variability for total bile acids was higher than for other parameters, the values were within the range expected for healthy animals. **Conclusion:** Clinical chemistry indicators of liver injury in rats and dogs do not demonstrate toxicologically significant diurnal changes. **Impact Statement:** The results of this comprehensive study contribute to the understanding of variability in clinical chemistry indicators of liver injury over a 24 hour time course for naïve rats and dogs.



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Wnt Inhibition Effects on Bone Formation in Preclinical Models Predicts Effects in Patients

Category: Systemic/Organ-Specific Toxicologic Pathology

Rene Meisner¹, Jie Wei¹, Jalpa Shah¹, Jennifer Cain¹, Tim Hoey¹

¹OncoMed Pharmaceuticals, Redwood City, CA, USA

Introduction: The Wnt pathway plays a central role in the regulation of stem cells and is implicated in many human cancers. We have developed two molecules that inhibit Wnt signaling – anti-FZD (OMP-18R5, vantictumab) and FZD8-Fc (OMP-54F28). These agents have been evaluated in multiple safety and efficacy models, and have been associated with decreased formation of trabecular bone.

Experimental Design: Microscopic evaluation of femurs from mice treated with OMP-18R5, OMP-54F28 or control antibody, with and without zoledronic acid administration, was conducted.

Methods: NOD.SCID mice were treated with Wnt inhibitors in various efficacy studies. In one experiment, a single dose of zoledronic acid was given at the initiation of dosing. Routinely processed femurs were examined by light microscopy. Sera were evaluated for markers of bone formation and degradation.

Results: Dose and schedule dependent decreases in the amount of subchondral bone was observed in the femurs of mice treated with WNT inhibitors. A single IV administration of zoledronic acid to mice resulted in subchondral bone formation comparable to control mice.

Conclusion: Inhibition of Wnt/FZD signaling decreases trabecular bone formation in mice, and is consistent with observations in patients. Bisphosphonate administration may be protective against the catabolic effects of Wnt inhibition, allowing benefits of targeting Wnt signaling for the development of anti-cancer therapy.

Impact Statement: Rodents are a sensitive model for evaluating the effects of Wnt inhibition on bone homeostasis. Patients are monitored for increased bone turnover and can be safely managed with administration of zoledronic acid, when indicated.



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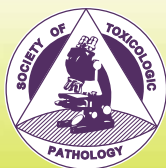
Ultrastructural Changes in Renal Papillary Duct Epithelium of Rats Treated with Trimethyltin Chloride

Category: Systemic/Organ-Specific Toxicologic Pathology

Kenichiro Kasahara¹, Hiroshi Edamoto¹, Mizuho Takagi¹, Ryo Ando¹, Kayoko Kudo¹, Rie Andoh¹, Kazutoshi Tamura¹

¹BoZo Research Center, Inc., Shizuoka, Japan

Introduction: Eosinophilic granules histologically observed in the renal papillary duct epithelium of rats treated with trimethyltin chloride (TMT) were examined ultrastructurally. **Experimental Design:** Nine-week-old female Crl:CD(SD) rats were orally administered a single dose of 10 mg/kg of TMT dissolved in distilled water. Animals were sacrificed at 1, 3, 6, 24 and 48 hours after treatment (HAT), and the kidneys were examined histologically and ultrastructurally. **Results:** Histologically, a small amounts of fine eosinophilic granules were observed in the cytoplasm of papillary duct epithelial cells at and after 3 HAT. The number of eosinophilic granules increased with time until 48 HAT. Ultrastructurally, multivesicular bodies/late endosomes and dilated cisternae probably of endoplasmic reticulum and/or Golgi apparatus were found in the cytoplasm of papillary duct epithelial cells at 1 HAT. At and after 3 HAT, the vesicles which contained multivesicular bodies/late endosomes, concentric membranous structures and glycogen granules increased in number with time. The vesicles were generally surrounded by a double-membrane, but some of them were not perfectly surrounded by the membrane. Sequestered materials in the vesicles were not degraded until 48 HAT, and secondary lysosomes and residual bodies were hardly detected throughout the experimental period. **Conclusion:** Eosinophilic granules histologically observed in the cytoplasm of renal papillary duct epithelial cells of rats treated with TMT were considered to be amphisomes judging from their ultrastructural characteristics. Amphisomes increased in number with time, and their contents were not degraded until 48 HAT.



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Investigation of CNS Findings Following Raxibacumab Treatment against Inhalation Anthrax in the New Zealand White Rabbit Model

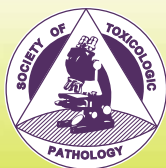
Category: Systemic/Organ-Specific Toxicologic Pathology

Maggie Dempster¹, Thi-Sau Migone², Sally Bolmer³, John Zhong⁴, Linda Hendey⁵, Dan Sanford⁵, Daphne Vasconcelos⁵

¹GlaxoSmithKline, King of Prussia, PA, USA, ²Igenica, Burlingame, CA, USA, ³Consultant, San Francisco, CA, USA, ⁴Biogen Idec, Cambridge, MA, USA, ⁵Battelle, Columbus, OH, USA

This work was performed in support of a contract with the US Department of Health and Human Services administered by the Biomedical Advanced Research and Development Authority to produce raxibacumab for the Strategic National Stockpile as a BioShield product. Studies sponsored by Human Genome Sciences (now acquired by GlaxoSmithKline).

Introduction/Objectives: Raxibacumab is a human IgG₁λ monoclonal antibody that binds PA (protective antigen) and inhibits PA binding to anthrax toxin receptors. Raxibacumab increases survival when administered prior to or after the onset of symptoms in anthrax spore inhalation in rabbits and monkeys. The purpose of this study was to assess the terminal pathology in both non-surviving and surviving rabbits after anthrax exposure and therapeutic treatment with raxibacumab. **Methods:** Parallel-group, blinded, randomized, GLP study in healthy rabbits. **Experimental Design:** Rabbits were administered a single IV vehicle or 40 mg/kg raxibacumab dose after detection of plasma PA, following 200 x LD₅₀ B. anthracis spore challenge. Both non-surviving and surviving rabbits (28 days post challenge) were subjected to gross and histopathological evaluation. **Results:** Survival for raxibacumab was significantly higher than vehicle (p < 0.0001). Findings consistent with anthrax were present in non-surviving rabbits. With the exception of increased CNS lesions (e.g. inflammation) in the raxibacumab group, all other lesions were more prevalent in the vehicle group. Raxibacumab-treated rabbits with the most severe lesions died later in their course of disease and IHC demonstrated presence of raxibacumab in CNS, when endogenous IgG was also present. Surviving rabbits (only raxibacumab-treated) exhibited no CNS lesions. **Conclusion:** Presence of raxibacumab in the CNS and time course of infection suggests that raxibacumab accesses the brain due to altered BBB and does not precede anthrax infection or exacerbate it. **Impact statement:** Raxibacumab has been shown to increase survival. Increased CNS inflammation is not evidence of a safety finding for raxibacumab.



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The Histomorphometric Feature of the Femoral Growth Plate in Young Rats under Two-Week Administration of 5-Fluorouracil and Dietary Restriction

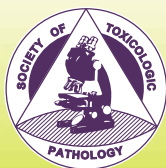
Category: Systemic/Organ-Specific Toxicologic Pathology

Chihiro Noguchi¹, Kenta Matsue¹, Yutaka Nakanishi¹, Fumiko Asanuma², Hiroto Miyata³, Minoru Sasaki¹, Syunsuke Tsutsumi¹, Yasushi Sato¹

¹Drug Safety and Pharmacokinetics Laboratories, Taisho Pharmaceutical Co., Ltd., Saitama, Japan,

²Regulatory Affairs Division, Taisho Pharmaceutical Co., Ltd., Tokyo, Japan, ³Prescription Drug Pharmacovigilance Division, Taisho Pharmaceutical Co., Ltd., Tokyo, Japan

Introduction: The purpose of this study was to evaluate the effect of 14-day decreased food consumption on growth plate with histomorphometric analysis in rats. **Experimental Design and Methods:** Six-week-old male rats were divided into control, 5-Fluorouracil (5-FU), and dietary restricted groups receiving the same amount of food in the 5-FU group, and 5-FU and dietary restriction groups were treated for 14-day (Miyata et al, *J Toxicol Pathol* 2009). The sections of the femur in this study were evaluated histomorphometrically in all groups by measuring the widths and number of chondrocytes per column of proliferative and hypertrophic zones in growth plate. **Results:** Decreases in the widths and number of chondrocytes in proliferative zone were observed in both of the 5-FU and dietary restricted groups with a similar manner when compared to controls. **Conclusion:** These findings suggest that 14-day dietary restriction caused biologically significant decreases in the widths and number of chondrocytes of proliferative zone, and the similar decreases found in the 14-day 5-FU treatment were also affected by diet restriction rather than direct drug effect, while 5-FU is known to affect growth plate directly in higher single doses. **Impact statement:** The effect of decreased food consumption should be taken into consideration when evaluating toxicologic effects on growth plate of the bone in rats, in terms of the widths and number of chondrocytes of proliferative zone, especially when evaluating some drugs which are expected to have possible effects on inhibiting the proliferation of growth plate.



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Immunohistochemistry on KIM-1 and BrdU Labeling of Cell Injury and Proliferation from 6-Month Repeated Dose Oral Toxicity Study of Canagliflozin in the Male Rat

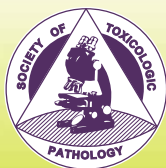
Category: Systemic/Organ-Specific Toxicologic Pathology

Jing Ying Ma¹, Sandra De Jonghe², Jim Proctor³, Mark D. Johnson³, Rao N. V. S. Mamidi³, Calvert Loudon⁴, Sandra Snook¹

¹Janssen R&D, LLC, LaJolla, CA, USA, ²Janssen R&D, LLC, Beerse, Belgium, ³Janssen R&D, LLC, Raritan, NJ, USA, ⁴Janssen R&D, LLC, Spring House, PA, USA

Introduction: The purpose of this study was to assess the potential effect of canagliflozin on cell proliferation or damage in the kidney when administered male-rats fed a standard-diet or high fructose-diet, for 6 months. The dose of 100mg/kg was selected for this study because it induced renal tubular tumors in a rat 2-year carcinogenicity study. Further, it was shown in a two-week study to induce carbohydrate malabsorption and secondary increased urinary calcium excretion in rats fed a standard-diet. It is hypothesized that these rat tumours can be attributed to carbohydrate malabsorption.

Experimental Design: There were five groups in this study, canagliflozin administered once daily, orally to rats fed a standard diet (\pm 100 mg/kg) or high fructose diet (\pm 100 mg/kg plus a group at 65 mg/kg) for 6 months. **Methods:** Formalin-fixed, paraffin-embedded kidney sections were immunolabeled with KIM-1 or BrdU. Renal tubular cell injury and proliferation were compared across diet and treatment groups using labeling index for KIM-1 /BrdU, respectively. **Results:** Our results showed in rats that canagliflozin at 100 mg/kg/day and fed a standard-diet resulted in statistically significant increased BrdU and TIM-1 labeling in the renal cortex/OSOM when compared to the standard-diet group. This response was diminished with the fructose-diet. **Conclusion:** Our findings supported the hypothesis that the renal tumors seen in rats are secondary to renal tubular injury induced by carbohydrate malabsorption. **Impact statement:** As carbohydrate malabsorption and hypercalciuria are not seen in humans administered canagliflozin, this tumor response in rats is considered species selective.



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Interpretation of Rodent Respiratory Histopathology Following Repeat Inhalation Exposures to Agrochemical Aerosols with Irritant Properties

Category: Systemic/Organ-Specific Toxicologic Pathology

Ronnie Chamanza¹, Jayne A Wright¹, Pramila Singh², Paul Hinderliter², Douglas Wolf², Richard Lewis¹
¹Syngenta, Jealott's Hill, Bracknell, Berkshire, UK, ²Syngenta, Greensboro, NC, USA

Legislation that regulates crop protection products allows the U. S. EPA to request repeat inhalation toxicity studies for non-volatile agrochemicals. However, for those with inherent irritant properties, the added value of such studies is unclear. Like most inhaled irritants, they produce site-specific lesions in the upper respiratory tract, mostly comprising of squamous metaplasia. A series of repeat inhalation exposures to different non-volatile agrochemical aerosols (MMAD = 1-3 μ m), ranging from 1 to 4 weeks in duration were conducted in rats. In each study, concentration-related squamous metaplasia was observed in the rostral nasal cavity and ventrolateral larynx. In the nasal cavity, the ventro-lateral meatus lined by a transitional epithelium was typically affected, with a rostro-caudal (decreasing) gradient in the incidence and severity. In the larynx, the epithelium above the base of the epiglottis, also an area of transition from squamous to respiratory epithelium was affected. This site-specificity is considered to be related to local airflow, breathing pattern, the anatomy and predicted site of impaction, the physico-chemical nature of the inhaled substance, aerosol droplet size, and the susceptibility of the affected tissues. The minimal to mild squamous metaplasia described is considered a non-specific and potentially reversible adaptive response to irritant properties of a compound that may be enhanced when very fine aerosols are generated. Such irritant properties are typically characterized during product development using other less labor and animal-intensive methods which would readily predict the likelihood of such a response following repeated exposures, without the need to perform subchronic inhalation studies.



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Female SDT Fatty Rat Shows Non-Alcoholic Steatohepatitis (NASH)-Like Hepatic Lesions

Category: Systemic/Organ-Specific Toxicologic Pathology

Katsuhiko Miyajima¹, Takeshi Ota², Yoshiaki Katsuda², Yukihiro Ishii², Kochi Kakimoto¹, Yuzo Yasui¹, Yusuke Kemmochi¹, Akiko Anagawa-Nakamura¹, Kaoru Toyoda¹, Eriko Tanai¹, Akemi Takahashi¹, Yoshifumi Miyakawa², Toshiyuki Shoda¹

¹Japan Tobacco Inc. Central Pharmaceutical Research Institute, Hadano, Kanagawa, Japan, ²Japan Tobacco Inc. Central Pharmaceutical Research Institute, Takatsuki, Osaka, Japan

Introduction: Obesity and type 2 diabetes are a well-established risk factor for many chronic disorders such as non-alcoholic steatohepatitis (NASH). Spontaneously Diabetic Torii-Lepr^{fa} (SDT-fatty) rat shows several metabolic syndrome features, obesity hyperglycemia and hyperlipidemia from 6 weeks of age. However, the pathophysiology of the liver in SDT-fatty rat has not been reported in detail. **Methods:** Female SDT-fatty rats and age-matched SD rats were allowed ad libitum feeding. Body weight and biochemical parameters, such as serum glucose, TG, T-CH, ALT and AST levels were evaluated at 8 weeks of age in the non-fasting state, and at 8-week intervals from 8 to 40 weeks of age. Histopathological examinations of the liver were performed by using the specimens stained with HE, Sirius red, toluidine blue (TB) and immunohistochemistry for ED-1. **Results:** SDT-fatty rats showed significantly increased body weight compared with SD rats. Serum glucose, TG and T-CH levels were significantly higher in SDT-fatty rats than in SD rats. Serum AST and ALT levels in the SDT-fatty rats significantly elevated from 8 weeks of age. Histopathologically, severity of hepatosteatosis accompanied by the inflammation increased from 8 weeks of age, and fibrosis began to occur from 32 weeks of age. In the liver of SDT-fatty rats, increased staining intensities for fibrosis (Sirius red), macrophages (ED-1) and mast cells (toluidine blue) were observed at 32 weeks of age. **Conclusion:** Based on this study, SDT-fatty rat is a good animal model to study NASH with type 2 diabetes and obesity, for which few models currently exist.



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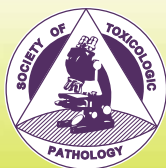
Histopathological Effects of Enrofloxacin in the Chicken Gastrocnemius Tendon

Category: Systemic/Organ-Specific Toxicologic Pathology

Uriel Blas-Machado¹, Sarah Quattlebaum¹, Deborah Keys¹, Jian Zhang¹, Jaroslava Halper¹

¹University of Georgia, Athens, GA, USA

Introduction: The association between administration of fluoroquinolones and degenerative tendon disease was recognized during the early 1980's. The main objective of the current study was to confirm the results of our previous in vitro study, which used avian embryonic tenocytes, in an in vivo model for degenerative tendon disease by a fluoroquinolone antibiotic. **Experimental Design:** From a total of 60, 1 day-old, male Avian reovirus-free White Leghorns chickens were used as follows, 48 chickens were exposed to enrofloxacin either in drinking water or by injection for 7 consecutive days (experimental days 0 - 7), and 12 chickens were exposed to enrofloxacin-free drinking water and/or injected with physiological saline solution to serve as controls. **Methods:** Chickens were necropsied at experimental day 0, 14, and 42. The response of the gastrocnemius tendon (GT) to enrofloxacin was followed by clinical observation, necropsy, histopathology, and immunohistochemistry for decorin and collagen I. **Results:** Gross lesions were absent in all birds. The GT enthesis of enrofloxacin-treated chickens developed significant degenerative changes by day 14 regardless of the day sampled, dose, or treatment route. Immunohistochemical detection of decorin and collagen I was diminished in injured areas. Findings were consistent with our previous in vitro findings. **Impact Statement:** Our observations indicate that the chicken serves as an acceptable in vivo chemical model for the study of fluoroquinolone GT degeneration.



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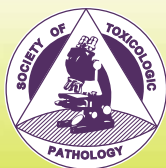
Conduritol β -Epoxide-Induced Neuroinflammation in C57Bl/6J Mice: A Translational Drug Discovery Tool for Neuropathic Gaucher Disease

Category: Systemic/Organ-Specific Toxicologic Pathology

Dinesh Bangari¹, John Marshall¹, Errin Roberts¹, Amy Allaire¹, Eva Budman¹, John Leonard¹, Beth Thurberg¹

¹Genzyme, Framingham, MA, USA

Introduction: Gaucher disease (GD) is a lysosomal storage disorder caused by genetic deficiency of glucocerebrosidase (Gba), which leads to progressive lysosomal accumulation of glucosylceramide (GL-1). Neuropathic forms of GD (Type 2/3; GD 2/3) are not amenable to the currently available enzyme replacement therapy for GD type 1. A reliable translational model will facilitate drug discovery and development for type GD2/3. **Experimental Design:** Here we present a mouse model of neuroinflammation generated by systemic dosing of conduritol β -epoxide (CBE), a covalent inhibitor of Gba. Three to Four week old female C57Bl/6J mice were administered CBE via intraperitoneal injection @100mg/kg daily for 7 weeks. **Methods:** Mice were monitored for neuromotor deficits via behavioral assays such as rotarod and qualitative hind-limb splay test. At the study end, tissue samples were collected for lipid analysis by mass spectrometry assays. Formalin-fixed, paraffin-embedded samples of brain were used for histopathology and immunohistochemistry. **Results:** Compared to saline-injected control mice, CBE-injected mice exhibited distinct neuromotor deficits and significantly increased levels of GL-1 and Lyso-GL-1 in the brain and liver. CBE-injected mice also revealed widespread gliosis /neuroinflammation in cerebral cortex, striatum, thalamus, and substantia nigra. Quantitative assessment revealed significantly increased CD68 and GFAP immunostaining in CBE-injected mice. Eosinophilic inclusions/structures suggestive of degenerative axons/aggregated proteins were observed in the cortex, striatum, and substantia nigra. **Conclusion:** CBE-dosing of female C57Bl/6J mice resulted in neuroinflammation and other histologic changes reminiscent of GD 2/3. **Impact Statement:** Overall, this model offers an attractive investigative and drug discovery tool for neuropathic GD and related disorders.



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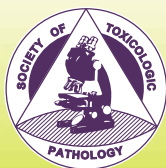
Normal Anatomy, Histology and Spontaneous Pathology of the Nasal Cavity of the Cynomolgus Monkey

Category: Systemic/Organ-Specific Toxicologic Pathology

Ronnie Chamanza^{1,2}, Ian Taylor³, Colin Hill³, Mark Swann³, Joel Goodchild³, Kane Goodchild³, Jane Schofield³, Mark Aldous³, Michela Gregori², Vasanthi Mowat²

¹Syngenta Limited, Jealott's Hill, Bracknell, Berkshire, UK, ²Huntingdon Life Sciences, Huntingdon, Cambridgeshire, UK, ³Huntingdon Life Sciences, Eye, Suffolk, UK

Although nonhuman primates are routinely used for inhalation toxicity studies, there is little published information on the normal anatomy and background pathology of nasal cavities in this species. We examined nasal cavities of 114 control Cynomolgus monkeys (57 males, 57 females) from eleven inhalation studies of 2 - 13-week duration conducted between 2008 and 2013, in order to determine the incidence and range of spontaneous pathology findings. Nasal sections from non-inhalation study animals were also examined grossly for detailed anatomic features and accessory structures. Compared to other laboratory animals, gross unique features of the monkey nose include; a relatively simple nose that is completely divided into two symmetrical passages, two simple dorso-ventrally stacked turbinates, and the presence of paired maxillary sinuses and nasopalatine ducts. Vomeronasal organs and a transverse lamina (that separates the olfactory region from the nasopharynx) are absent. The epithelial lining is relatively high, the respiratory epithelium rests on a very thick basal lamina, and olfactory tissue is restricted to a small dorso-posterior area. Squamous and transitional epithelia occupy the vestibule and dorso-anterior parts. The incidences of spontaneous findings were relatively high and were distributed equally between sexes. Findings resembling compound-induced lesions were also encountered and included; inflammatory lesions on the anterior lateral walls and the dorsal olfactory epithelium, luminal exudate, scabs on the transitional/squamous epithelial junction, squamous and respiratory epithelial hyperplasia, squamous metaplasia, mucous cell hyperplasia, and olfactory epithelial degeneration and respiratory metaplasia. This information is considered useful for the interpretation of histopathology results from inhalation studies.



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Background Incidence of Spontaneous Adrenal Gland Lesions of Control Charles River CD-1 Mice (CrI: CD-1(ICR) BR) Used in 104-Week Toxicity Studies

Category: Systemic/Organ-Specific Toxicologic Pathology

Claudio Petterino¹, Stuart Naylor¹, Sydney Mukaratirwa¹, Alys Bradley¹

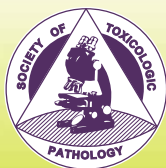
¹Charles River, Ltd., Tranent, UK

Introduction: The aim of this study was to provide the range and incidences of spontaneous lesions of the adrenal glands in control CD-1 mice from 104-week studies carried out at Charles River, Edinburgh.

Experimental Design: Data were collected retrospectively from control CD-1 mice over a period of 12 years (1998-2010) giving a total of 1205 control animals (598, males; 607, females).

Methods and Materials: All control animals were obtained from groups of animals that had been sham dosed with an appropriate vehicle. Tissues were examined histopathologically, and the findings were entered directly into a validated computerized database. Each study was subjected to an internal peer review and all data reviewed by the Quality Assurance Department at Charles River, Edinburgh. **Results:** The most common non-proliferative lesions in male animals were cortical cell hypertrophy (20.7%), cortical atrophy (9.8%), and pigment deposition (3.6%), and in females pigment deposition (6.4%), cortical atrophy (2.1%), and amyloid deposition (1.8%). The most common proliferative lesions in male animals were subcapsular cell hyperplasia (38.6%), subcapsular cell tumour (9.3%), and focal cortical cell hyperplasia (8.0%), and in females subcapsular cell hyperplasia, (74.9%), subcapsular cell tumor (2.9%), and focal cortical cell hyperplasia (1.4%). **Conclusions:** To the best of our knowledge this is the most comprehensive study of the incidences of background lesions in adrenal gland in control CD-1 mice.

Impact Statement: The results represent a useful source for the incidence of spontaneous findings in adrenal glands in control CD-1 mice from 104-Week carcinogenicity studies.



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Mesenchymal Tissue Formation in Renal Tubule Lumen of Cyclosporine-Induced Acute Kidney Injury in a Cynomolgus Monkey

Category: Systemic/Organ-Specific Toxicologic Pathology

Kiyonori Kai¹, Satoko Sato¹, Kayoko Ishikawa¹, Takashi Yamaguchi¹, Munehiro Teranishi¹, Wataru Takasaki¹

¹*Daiichi Sankyo Co., Ltd., Tokyo, Japan*

Introduction: Kidney exhibits remarkable ability to survive injury. It is still not clear whether cellular repair in adult kidney is carried out by specialized renal progenitors residing in specific niches or by self-duplication/de-differentiation of mature cells. **Experimental Design:** A male cynomolgus monkey (9 years old) was given 60 mg/kg of cyclosporine subcutaneously for 6 days (originally 14 days). **Methods:** Urinalysis and blood biochemistry were conducted in pre-dose and dosing period, and the monkey was euthanized due to deterioration of general condition on Day 6. Routine light and electron microscopy of the kidney was performed, and the kidney was subjected to PAS reaction, silver stain and immunohistochemistry using vimentin, WT-1, aquaporin 2, calbindin-D28k and OCT4 antibody. **Results:** Urinalysis and blood biochemistry represented acute kidney injury and oliguria was noted on Day 6. In histopathology of the kidney, severe vacuolation of proximal tubular epithelium and slight dilatation of distal tubule were observed. Interestingly, cluster of polygonal or spindle cells were seen in the tubular lumen lined by flattened epithelial cells in the cortex. The cells possessed cell process and showed retiform or glomerulus-like structure. However, reticulum fiber was not noted in the structure and PAS positive material was observed at enclosed structure of the cells. The cells showed positive reaction to vimentin and OCT4, and occasionally stained by calbindin-D28k. **Conclusion:** Epithelial-to-mesenchymal transition was noted in cyclosporine-induced acute kidney injury in a cynomolgus monkey. **Impact statement:** Renal cell flexibility acquired by cyclosporine treatment may reveal new insight of multi-potential of somatic cells.



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Exogenous Human Thioredoxin-1 Ameliorates Acetaminophen-Induced Acute Liver Toxicity and Liver Failure through Reducing Peroxynitrite and Inhibiting Degradation of Endogenous Thioredoxin-1

Category: Systemic/Organ-Specific Toxicologic Pathology

Byungwoo Lee¹, Jonghyeok Ko¹, Seonyong Kim¹, Byungil Yoon¹

¹Kangwon National University, Chuncheon, Gangwon-do, Republic of Korea

Introduction: Thioredoxin-1 (Trx-1) has multiple functions including anti-oxidation, anti-inflammation and anti-apoptosis, but excessive oxidative stress can induce degradation of Trx-1, resulting in cell injury. Overdose of acetaminophen (APAP) causes hepatotoxicity by generation of reactive nitrogen species, mitochondrial dysfunction, and DNA damage. **Experimental design:** For preventive study, C3H mice were pretreated with human recombinant Trx-1(rhTrx-1; 10, 50 or 100mg/kg), followed by administration of APAP, and then sacrificed at 6h. To see the favorable effect of rhTrx-1 for survival, the mice were treated with rhTrx-1(10mg/kg) 30 min before oral administration of lethal dose of APAP (400 mg/kg b.w.) and then monitored up to 72h. **Methods:** After gross examination, hematoxylin and eosin-stained livers were prepared for histologic examination. The necrotic areas were quantitated by measuring the areas of necrosis using image analysis. TUNEL assay and immunohistochemistry for 3-nitrotyrosine were carried out to detect the apoptotic and peroxynitrite-affected cells, respectively. Western blotting was also performed to quantitate peroxynitrite and Trx-1 level in the liver. **Results:** Pretreatment of rhTrx-1 markedly reduced the APAP-induced hepatocytic necrosis and apoptosis. In addition, exogenous rhTrx-1 decreased the lethality of APAP up to 50%. Those results were corresponding to the inhibitory effect of rhTrx-1 against the nitrotyrosine production following APAP treatment. Exogenous rhTrx-1 also maintained the effective level of Trx-1 in liver that was markedly decreased by APAP. **Conclusion:** rhTrx-1 ameliorates APAP-induced liver injury through reducing peroxynitrite production and inhibiting degradation of endogenous Trx-1. **Impact statement:** Exogenous rhTrx-1 can be protective against APAP-induced hepatotoxicity and liver failure.



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Comparative Histology of Mouth Mucosae (Sublingual Region)

Category: Systemic/Organ-Specific Toxicologic Pathology

Catherine Thirion-Delalande¹, Cécile Fisch², Roy Forster¹, Bernard Palate¹

¹CiToxLAB, Evreux, France, ²Stallergenes S.A., Antony, France

Introduction: This study was undertaken to compare the sublingual mucosae from several laboratory animal species and humans.

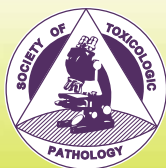
Experimental design: Mucosae (including both epithelium and lamina propria) from the inferior surface of the tongue, cheek and/or mouth floor, were described for 8 laboratory animal species and humans.

Methods: Mouth mucosae were collected from mouse, rat, hamster, guinea pig, rabbit, dog, macaque monkey and minipig (n=3 for each). One human tongue was obtained to compare with these animal tissues. Samples were fixed in formalin, processed, trimmed and stained with hematoxylin/eosin, Periodic Acid Schiff, diastase/Periodic Acid Schiff and/or Masson trichrome.

Results: In humans, the inferior surface of the tongue is lined by a stratified, squamous, non keratinized epithelium which is closely resembled by that of non-human primates. The oral mucosae of minipigs, dogs and rabbits present similar histological features and are comparable. All the rodent species studied have thinner, keratinized mucosae. The thickness of the oral epithelium and lamina propria, as well as the grade of rete ridges/papillae, are more or less proportional to the size of the species. Glycogen is seen in the superficial layers of human, non-human primate and minipig mucosae.

Conclusion: Non rodent (monkey, minipig, dog and rabbit) and human mucosae have comparable histological features. Rodent oral mucosae are thinner and keratinized.

Impact statement: Based upon this qualitative histological evaluation, the mouth mucosae of non-human primates and minipigs were found to be closest to human mouth mucosae.



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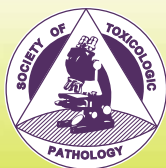
Non-Neoplastic Histologic Cutaneous Background Lesions in Göttingen Minipigs and Findings at Treatment Control Sites

Category: Systemic/Organ-Specific Toxicologic Pathology

Charlotte Hollinger¹, Keith Nelson^{2,1}

¹Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA, ²MPI Research, Mattawan, MI, USA

Introduction: Göttingen minipigs are increasingly used as non-rodent models, particularly in studies of dermal toxicity. Recognition of cutaneous background lesions, and influences of vehicle compounds and iatrogenic factors, is necessary to reliably identify and describe test article effects. The purpose of this study was to review and compare background lesions occurring at untreated and treatment-control skin sites in control Göttingen minipigs over a 10-year period at MPI Research. **Methods:** Histologic findings in the skin of control animals were tabulated from 40 studies (29 dermal) with durations from 2 to 36 weeks. The studies involved 211 female and 205 male minipigs, with 415 untreated and 279 treatment-control site samples. The treated site samples had vehicle and/or mechanical interventions (e.g., shaving, bandaging) mimicking animals dosed with test articles. **Results:** The most prevalent histologic changes in untreated site samples were exudation/crusting (4.6%), perivascular inflammation/infiltration (4.1%), and subacute/chronic inflammation (2.9%). The most prevalent histologic changes in treatment control sites were epidermal hyperplasia (14.4%), hyperkeratosis (8.2%), and subacute/chronic inflammation (6.3%). Treated skin tissues had an eight-fold increase in hyperplasia, seven-fold increase in lymphohistiocytic infiltration, and five-fold increase in hyperkeratosis. Fungal organisms and fibrosis were only recorded for treated control samples. **Conclusion:** Cutaneous studies in Göttingen minipigs must be interpreted with awareness of spontaneous lesions in the skin of these animals and the confounding effects of test-article independent procedure-related factors. **Impact statement:** This study provides a focused review of background findings in the skin of the Göttingen minipig model for dermal toxicity testing.



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Temporal Patterns of Candidate Biomarkers of Vascular Injury in the IL2 Rat Model

Category: Systemic/Organ-Specific Toxicologic Pathology

Cristina Bertinetti-Lapatki¹, Natalie Keirstead², Denise Knapp¹, Igor Mikaelian³, James R Turk⁴, Bradley E. Enerson⁵, Holly W. Smith⁶

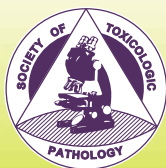
¹Hoffman-La Roche, Inc., Basel, Switzerland, ²Astra-Zeneca, Waltham, MA, USA, ³Abbvie, Inc., Worcester, MA, USA, ⁴Amgen, Inc., Thousand Oaks, CA, USA, ⁵Pfizer, Inc., Groton, CT, USA, ⁶Eli Lilly, Indianapolis, IN, USA

BACKGROUND/PURPOSE: Drug-induced vascular injury is a major cause of termination of clinical drug candidates. The purpose of this study was to evaluate candidate biomarkers of DIVI in rats administered recombinant IL2 (rIL2).

METHODS: Male Wistar Han rats were administered rIL2 at 0.42 mg/kg/day (first day) and 0.36 mg/kg/day subsequently for 3 or 5 days, followed by a 7-day recovery period. The endpoints included routine clinical pathology, histopathology using PSTC's lexicon, plasma miRNAs, and serum biomarkers.

RESULTS: The administration of rIL2 was associated with time-dependent changes that included endothelial hypertrophy/hyperplasia (HH), perivascular inflammation, and vascular smooth muscle cell hyalinization, with partial recovery after 7 days off-dosing. These findings correlated well with multiple candidate biomarkers of DIVI, including angiopoietin 2 (ANGPT2), E-selectin (SELE), chemokine (C-X-C motif) ligand 1 (CXCL1), lipocalin 2 (LCN2), monocyte chemoattractant protein-1 (MCP1), tissue inhibitor of metalloproteinase 1 (TIMP1), and vascular endothelial growth factor A (VEGFA).

CONCLUSION: The proposed candidate biomarkers adequately identified the early stages of DIVI as captured by a vascular-centric lexicon. The validation of these candidate biomarkers is in progress and will require their evaluation in animal models with different underlying mechanisms of DIVI and in models of tissue-specific toxicity with no, or minimal, vascular involvement.



Poster Abstracts

33rd Annual Symposium



TRANSLATIONAL PATHOLOGY:
Relevance of Toxicologic Pathology
to Human Health

Washington, DC

Marriott Wardman Park Hotel

June 22–26, 2014

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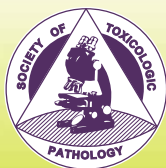
Renal Epithelial Hyperplasia Caused by Urinary Crystals of a Novel GKA and Its Metabolites after Acute Dosing in Sprague Dawley Rats

Category: Systemic/Organ-Specific Toxicologic Pathology

ES Tien³, NE Everds², JR Turk¹, MP Nguyen², Q Ye⁴, P Cao⁴, L Jim⁴, KA Samoya⁴, PD Schnier⁴, RC Kelly⁴, JF Schroeder¹, CA Afshari¹

¹Amgen, Inc., Thousand Oaks, CA, USA, ²Amgen, Inc., Seattle, WA, USA, ³Amgen, Inc., Cambridge, MA, USA, ⁴Amgen, Inc., South San Francisco, CA, USA

Renal toxicity caused by crystal formation in the urine is well-established for some drugs, industrial chemicals, food additives, and naturally occurring toxins but toxicity typically requires long term dosing to cause measurable changes in urinary system morphology and/or function. Compound A, a novel glucokinase activator (GKA), caused crystal formation in the urine with accumulation in the collecting ducts accompanied by renal transitional epithelial hyperplasia with neutrophil infiltration after only 4 consecutive daily doses in female Sprague Dawley rats. A second study dosing Compound A at 600mg/kg/day for 1, 2 or 4 consecutive days was performed to explore the unusually rapid progression of the hyperplasia and identify the crystals. Findings after a single dose included crystals in urine and kidneys and clinical pathology changes indicating inflammation and decreased renal function. Additional findings after 2 doses included renal transitional epithelial hyperplasia with neutrophil infiltration. No changes were observed in urinary bladder epithelium. Crystals in the kidneys were identified as Compound A and two major metabolites by MALDI mass spec imaging. Urinary system findings observed with Compound A have not been seen with other internally tested GKAs of similar structure nor GKAs reported in the literature, suggesting that it is unrelated to the biology of glucokinase activation. The renal toxicity caused by Compound A highlights possible rapid onset of such effects and acute toxicity studies could benefit from additional endpoints to more clearly define the nature of such findings if the potential exists for renal toxicity to occur.



Poster Abstracts

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Drug Candidate-Induced Changes in the Thyroid Gland: Contrasting Case Studies

Category: Systemic/Organ-Specific Toxicologic Pathology

Joan Lane¹, Doriana Froim¹, Daniel Aleksandrowicz¹, Jeffrey Horrigan¹, Katie Zokowski¹, Ken Loveday¹, David Peters¹, Evelyne Polack¹

¹Biogen Idec, Inc., Cambridge, MA, USA

Introduction: While xenobiotics that depress thyroid function by inhibition of thyroid hormone synthesis or enhancement of peripheral metabolism are fairly common, those that stimulate thyroid hormone synthesis are rare. Recently, we identified histomorphologic and functional thyroid changes associated with lead and follow on compounds for two preIND-stage programs. Program 1 targeted a tyrosine kinase expressed in lymphoid and myeloid cells; Program 2 targeted a G protein-coupled receptor with a broad expression profile. Findings indicated thyroid suppression in Program 1 and thyroid stimulation in Program 2. **Experimental Design:** For Program 1, 28-day oral repeat dose toxicology studies for the lead and 14-day dose range finding studies for the backup compound were completed in rats and dogs. For Program 2, 28 day oral repeat dose toxicology studies for both lead and back up molecules were completed in rats and cynomolgus monkeys. **Methods:** Standard toxicologic end points, serum Thyroid Stimulating Hormone (TSH), Triiodothyronine (T3), and Thyroxine (T4) were evaluated in 28 day studies. **Results:** For Program 1, increased thyroid weight in rats, increased TSH in rats and dogs, and decreased T4 in rats were observed. T3 was unchanged in both species. For Program 2, increased thyroid weight, elevated T3 and T4, and decreased TSH were noted in monkeys, but not in rats, and the pattern was conserved between lead and back up. **Conclusion/Impact statement:** Histopathologic changes in the thyroid correlating with the hormonal effects are described and the investigative activities and impact for each program are discussed.



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Characterization of a Minipig Model of Gastrointestinal Acute Radiation Syndrome Using Total Body Irradiation and Partial Body Irradiation: A Focus on Intestinal Pathology

Category: Systemic/Organ-Specific Toxicologic Pathology

Julius Haruna¹, Wieslaw Wierzbicki², Mylene Pouliot¹, Leanne Bassett¹, Alexis Ascah¹, Simon Authier¹
¹CiToxLAB North America, Laval, QC, Canada, ²Centre Vétérinaire DMV, Lachine, QC, Canada

Introduction: The gastrointestinal tract (GIT) of minipigs is similar to that of humans in transit time, adaptation to omnivore diet and humidity content of feces which impacts the bacterial flora. The minipig is a potentially valuable model for evaluating GIT-Acute Radiation Syndrome (ARS) treatments. We designed a set-up for total body irradiation (TBI) and partial body irradiation (PBI) in conscious minipigs.

Experimental Design: One male Gottingen minipig was assigned to each of three groups. Group 1 served as non-irradiated control. Group 2 (PBI) and 3 (TBI) were exposed to gamma radiation from a Co⁶⁰ source (Theratron1000).

Methods: Animals were irradiated with a single dose up to 12Gy, delivered in 2 lateral fractions at a dose rate of 50cGy/min. Dosimetry measurements were performed using a mini-pig acrylic phantom, a solid water phantom with Markus and Farmer ionization chambers. Cerrobend shielding (9 cm) provided attenuation of the dose delivered to the pelvic legs of the PBI minipig. Pigs were euthanized and necropsied following 7 days of observation. Small intestines, colon, rectum sternum and abnormal findings were processed and evaluated microscopically.

Results: Emesis and anorexia were consistently observed after radiation. At necropsy, dark red areas/focus consistent with hemorrhages were observed in the GIT. Major GIT microscopic changes included atrophy/loss of crypts, villous atrophy, crypt degeneration/regeneration, erosions/ulcerations and hemorrhages. Hematopoietic hypocellularity was noted in the bone marrow.

Conclusion: This report documents characteristic irradiation-related GIT changes in minipigs following PBI and TBI. Thus, the minipig model could be used to evaluate potential therapies for GIT-ARS.



105

Mechanisms of Acquired Resistance to Toseranib Phosphate (Palladia®) in Canine Mast Cell Tumor

Category: Oncology/Carcinogenesis

Charles Halsey¹, Daniel Gustafson¹, Barbara Rose¹, Robert Burnett¹, Dawn Duval¹, Anne Avery¹, Donald Backos², Philip Reagan², Douglas Thamm¹

¹Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO, USA,

²University of Colorado Health Science Center, Aurora, CO, USA

Introduction: Mast cell tumors (MCTs) are the most common skin tumors in dogs and exhibit variable biologic behavior. Mutations in the c-kit proto-oncogene are associated with the tumorigenesis of MCTs, resulting in growth factor-independent and constitutive phosphorylation of the KIT receptor tyrosine kinase (RTK). Small molecule inhibitors of KIT are an attractive therapeutic strategy for MCTs in dogs. Toseranib (TOC) phosphate (Palladia®) is a RTK inhibitor of KIT that has biological activity against MCTs. Despite these benefits, patients ultimately develop resistance. Therefore, there is a need to identify distinctive clinical and molecular features of resistance in this population.

Methods and Results: Canine C2 mastocytoma cell line contains an activating mutation in c-kit. Three TOC-resistant C2 sublines were established over seven months by growing cells in increasing concentrations of TOC. TOC inhibited KIT phosphorylation and cell proliferation in a dose-dependent manner in the treatment-naïve C2 line (IC₅₀ of <10 nM). In contrast, growth of the three sublines was resistant to inhibition by TOC (IC₅₀ > 1,000 nM) and phosphorylation of the KIT receptor was less inhibited compared to the TOC-sensitive C2 cells. Sequencing of c-kit revealed secondary mutations in the functional domains of the resistant sublines. Additionally, chronic TOC exposure resulted in c-kit and subsequent KIT overexpression in the TOC-resistant sublines compared to the parental line.

Conclusion and Impact: This study demonstrates the development of a canine model of acquired resistance to targeted therapy in tumors harboring a c-kit-activating mutation that may be used to investigate strategies to overcome drug resistance.



THE OHIO STATE UNIVERSITY

COLLEGE OF VETERINARY MEDICINE

**COLLEGE OF
VETERINARY MEDICINE
RESEARCH DAY**

7 APRIL 2016

**BOOK OF
ABSTRACTS**

PROGRAM

April 7, 2016

POSTER JUDGING

Graduate Student Posters

8:00 am – 10:30 am

(closed session – only open to
those being judged)

AWARDS PRESENTATION

Veterinary Medical Center Auditorium

12:00 pm

GRADUATE STUDENT and POST DOC PLATFORM PRESENTATIONS

Dr. Dimitria Mathys

Dr. Amanda Panfil

INAUGURAL RAINIER ENDOWED CHAIR LECTURE

Veterinary Medical Center Auditorium

immediately following the awards
and platform presentations

DR. G. Gilbert Cloyd

Chief Technology Officer of Procter & Gamble (Retired)

Ohio State CVM Alumnus

***“Veterinary Careers in Industrial Research and the
Importance of Private Sector Innovation”***

POSTER SESSION

1st and 2nd Floors – Vet Med Academic Building

11:00 am – 5:00 pm

CHAired BY

Dr. Patrick Green

ORGANIZED BY

Michele Morscher

Special thanks to Marc Hardman in the
College's Technology Services for printing the posters

POSTER JUDGING SESSIONS

Wednesday, April 6, 2016
2:00 – 5:00 pm
Undergraduate and
Veterinary Student Poster Judging

Thursday, April 7, 2016
8:00 – 10:30 am
Graduate Student Poster Judging

Thank you to the following faculty and guests for taking time
out of their busy schedules to judge 70 posters.

Jim Belknap

Andy Bowman

Prosper Boyaka

Rachel Cianciolo

Luciana da Costa

Miles Hall

Jim Hartke

Kate Hayes-Ozello

Ryan Jennings

Sanggu Kim

Stefan Niewiesk

Mike Oglesbee

Judy Radin

Dave Ralph

Yasuko Rikihisa

Thiru Selvanantham

Barb Wolfe



THE OHIO STATE UNIVERSITY

COLLEGE OF VETERINARY MEDICINE

Office of Research
and Graduate Studies

COLLEGE OF VETERINARY MEDICINE **RESEARCH DAY**

Awards Presentation, Graduate Student and
Post Doc Platforms, and
Inaugural Rainier Endowed Chair Lecture

Thursday, April 7th, 2016 Noon – 2 p.m.

Veterinary Medical Center Auditorium



Dr. G. Gilbert Cloyd

Chief Technology Officer
of Procter & Gamble
(Retired)

**“Veterinary careers in
industrial research and the importance
of private sector innovation”**

Poster judging:

April 6th, 2 – 5 p.m. for Professional Students

April 7th, 8 – 10:30 a.m. for Graduate Students

PLATFORM PRESENTATION

ENTEROBACTERIACEAE PRODUCING EXTENDED SPECTRUM β -LACTAMASES (ESBL) FROM WILD BIRDS IN OHIO. D.A. Mathys¹, B. A. Mathys², D.F. Mollenkopf¹, J.B. Daniels³, T.E. Wittum¹.

¹Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Ohio State University ²Department of Natural Sciences, Ohio Dominican University ³Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University

ESBLs confer bacterial resistance to critically important antimicrobials. Livestock are an important reservoir for the zoonotic food-borne transmission of resistant enteric bacteria. Our aim is to describe the potential role of migratory and resident wild birds in the epidemiology of ESBL mediated bacterial resistance on dairy farms. Using mist nets, we sampled wild migratory and resident birds either immediately adjacent to or 600 feet away from free stall barns on three Ohio dairy farms during 2014/2015 spring migration. Individual swabs were used to obtain both a cloacal and external surface swab from each bird. Additionally, wild ducks were sampled either live caught or hunter harvested from hunting preserves in 2014 and 2015. Samples were inoculated into MacConkey broth containing cefotaxime and inoculated onto MacConkey Agar with ceftiofur, cefepime, or meropenem to identify the *bla*_{CMY}, *bla*_{CTX-M}, and carbapenemase phenotypes, respectively. Six hundred and six birds were sampled, 14 (2.3%) of which harbored bacteria with the *bla*_{CMY} gene and 26 (4.3%) harbored bacteria with the *bla*_{CTX-M} gene from either their cloacal sample or from their external swab. There was no difference in the prevalence of either gene between migratory and resident birds. Prevalence of the *bla*_{CMY} was higher among birds sampled immediately outside the barns compared to those sampled 600 feet away. Six hundred and twenty seven ducks were sampled, with 44 (7%) harboring *bla*_{CMY} bacteria and 2 (0.3%) harboring *bla*_{CTX-M} bacteria. Our results suggest that wild birds can serve as mechanical and/or biological vectors for *Enterobacteriaceae* with resistance to extended spectrum cephalosporins. Birds live in close contact with dairy cows and their feed, therefore transmission locally from farm to farm is possible. Finding a similar prevalence in migratory and non-migratory birds suggests the potential for regional and intercontinental movement of these genes via birds.

Keywords: Antibiotic resistance, wildlife, vector, livestock

PLATFORM PRESENTATION

PRMT5 IS UPREGULATED IN HTLV-1-MEDIATED T-CELL TRANSFORMATION AND SELECTIVE INHIBITION ALTERS VIRAL GENE EXPRESSION AND INFECTED CELL SURVIVAL. A. Panfil, J. Al-Saleem, C. Howard, J. Mates, J. Kwiek, R. Baiocchi, and P. Green. Depts. of Veterinary Biosciences

Human T-cell leukemia virus type-1 (HTLV-1) is a tumorigenic retrovirus responsible for development of adult T-cell leukemia/lymphoma (ATLL). This disease manifests after a long clinical latency period of up to 2-3 decades. Two viral gene products, Tax and HBZ, have transforming properties and play a role in the pathogenic process. Genetic and epigenetic cellular changes also occur in HTLV-1-infected cells, which contribute to transformation and disease development. However, the role of cellular factors in transformation is not completely understood. Herein, we examined the role of protein arginine methyltransferase 5 (PRMT5) on HTLV-1-mediated cellular transformation and viral gene expression. We found PRMT5 expression was upregulated during HTLV-1-mediated T-cell transformation, as well as in established lymphocytic leukemia/lymphoma cell lines and ATLL patient PBMCs. shRNA-mediated reduction in PRMT5 protein levels or its inhibition by a small molecule inhibitor (PRMT5i) in HTLV-1-infected lymphocytes resulted in increased viral gene expression and decreased cellular proliferation. PRMT5i also had selective toxicity in HTLV-1-transformed T-cells. Finally, we demonstrated that PRMT5 and the HTLV-1 p30 protein had an additive inhibitory effect on HTLV-1 gene expression. Our study provides evidence for PRMT5 as a host cell factor important in HTLV-1-mediated T-cell transformation, and a potential target for ATLL treatment.

Keywords: ATLL; HBZ; HTLV-1; PRMT5; Tax; lymphoma; transformation

CLINICAL RESEARCH

CR - 1

MORPHOMETRIC DIFFERENCES OF COMPUTED TOMOGRAPHIC IMAGES BETWEEN THE CERVICAL VERTEBRAL FORAMEN OF GREAT DANES WITH AND WITHOUT SUBCLINICAL NEUROLOGIC DEFICITS. J. Arbogast, R. C. Da Costa DVM, MS, ACVIM. The Ohio State University, College of Veterinary Medicine, Columbus OH

CR - 2

ASSESSMENT OF BIOMARKERS OF PAIN AND ACTIVITY PATTERNS IN LACTATING DAIRY COWS DIAGNOSED WITH CLINICAL METRITIS. A.A. Barragan¹, J.J. Piñeiro¹, G. M. Schuenemann¹, P.J. Rajala-Schultz¹, D.E. Sanders², S. Bas¹.

¹Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210, ²Vaca Resources, Urbana, OH 43078

CR - 3

CLINICAL CHARACTERISTICS OF PRESUMPTIVE OR CONFIRMED FIBROCARILAGINOUS EMBOLIC MYELOPATHY (FCE): A SYSTEMATIC REVIEW OF 393 CASES FROM 1973 TO 2013. K. Bartholomew, S. Moore, K. Stover, and N. Olby. Dept. of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University (Bartholomew and Moore); and the Dept of Clinical Sciences, College of Veterinary Medicine, North Carolina State University (Stover and Olby).

CR - 4

COMPARISON OF ALBUMIN, COLLOID OSMOTIC PRESSURE, VON WILLEBRAND FACTOR, AND COAGULATION FACTORS IN CANINE CRYOPOOR PLASMA, CRYOPRECIPITATE, AND FRESH FROZEN PLASMA. C. Culler¹, M. Iazbik M¹, J. Guillaumin² ¹The Ohio State University Veterinary Medical Center. ² Department of Veterinary Clinical Sciences, Ohio State University

CR - 5

ANDROGEN AND PREGNANE RESPONSE TO STRESS IN CRITICALLY ILL FOALS. K. Dembek¹, K. Timko¹, L. Johnson¹, B. David², B. Barr³, K. Hart⁴, and R. Toribio¹, ¹Department of Veterinary Clinical Sciences, The Ohio State University, ²Hagyard Equine Medical Institute, ³Rood and Riddle Equine Hospital, ⁴College of Veterinary Medicine, University of Georgia

CR - 7

USE OF FOOD TO FACILITATE HANDLING OF DOGS DURING VETERINARY VISITS: EFFECTS ON GASTROINTESTINAL FUNCTION AND CLIENT PERCEPTIONS OF THE VISIT. M. Forman, T. Shreyer, T. Buffington, S. Barrett
The Ohio State University, Department of Veterinary Clinical Sciences

CR - 8

FIBROBLAST GROWTH FACTOR-23 IN CANINE CHRONIC KIDNEY DISEASE. L. Harjes, V. Parker, K. Dembek, L. Giovanni, M. Kogika, D. Chew, and R.E. Toribio. Department of Veterinary Clinical Sciences, The Ohio State University

CR - 9

LACTOFERRIN REDUCES MORTALITY IN PRE-WEANED CALVES WITH DIARRHEA. K. Harris and G. Habing. Department of Veterinary Preventive Medicine

CR - 10

COMPUTATIONAL FLUID DYNAMICS USING COMPUTED TOMOGRAPHY TO ASSESS AIRWAY RESISTANCE IN ENGLISH BULLDOGS. E.T. Hostnik¹, B.A. Scansen¹, R.E. Zielinski², and S.N. Ghadiali². ¹Department of Veterinary Clinical Sciences and ²Department of Biomedical Engineering, The Ohio State University, Columbus, OH 43210.

CR - 11

THE FGF-23/KLOTHO AXIS AND ITS ASSOCIATION WITH PHOSPHORUS, CALCIUM, VITAMIN D, PARATHYROID HORMONE, DISEASE SEVERITY AND OUTCOME IN HOSPITALIZED FOALS. A. Kamr^{1,4}, K.A. Dembek¹, B.E. Hildreth III¹, S.M. Reed², N.M. Slovis³, T.A. Burns¹, A. Zaghawa⁴, R.E. Toribio¹. ¹ Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH; ²Rood and Riddle Equine Hospital, Lexington, KY; ³Hagyard Equine Medical Institute, Lexington, KY; ⁴Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt

CR - 12

THE EFFECTS OF HYALURONAN ALONE OR IN COMBINATION WITH CHONDROITIN SULFATE AND N- ACETYL-D-GLUCOSAMINE ON LIPOPOLYSACCHARIDE-CHALLENGED EQUINE FIBROBLAST-LIKE SYNOVIAL CELLS. A. Kilborne, H. Hussein, A. Bertone. Department of Veterinary Clinical Sciences. College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.

CR - 13

ADRENOCORTICAL STEROID RESPONSE TO A HIGH DOSE OF ACTH IN HEALTHY AND CRITICALLY ILL FOALS. J.S. Minuto, K.A. Dembek, R.E. Toribio. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH

CR - 14

REGIONAL DIFFERENCE IN THE SPINAL EPENDYMAL LAYER OF NORMAL DOGS A Muir, SA Moore. Department of Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio

CR - 15

EVALUATION OF KINEMATIC MAGNETIC RESONANCE IMAGING IN DOGS WITH OSSEOUS-ASSOCIATED CERVICAL SPONDYLOMYELOPATHY. M. Provencher, A. Habing, S. Moore, L. Cook, G. Phillips, and R. da Costa. Departments of Veterinary Clinical Sciences and the Center for Biostatistics

CR - 16

CLINICAL STUDY OF ECHOCARDIOGRAPHIC VARIABILITY IN ESTIMATING PULMONARY ARTERY PRESSURE AND PULMONARY VASCULAR RESISTANCE IN DOGS. J. Rhinehart, K. Schober, B. Scansen, J. Bonagura. Depts. of Veterinary Clinical Sciences

CR - 17

BEHAVIORAL EFFECTS OF TRAZODONE ON HOSPITALIZED DOGS. S. Gilbert-Gregory, M.R. Rice, J. Stull, K. Proudfoot, M. Herron. Depts. of Veterinary Clinical Sciences and Preventative Medicine.

CR - 18

THE EFFECT OF INTRAVENOUS MAGNESIUM SULFATE (MgSO₄) ADMINISTRATION IN THE HORSE. S. Schumacher DVM, A. Bertone DVM, PhD, and R. Toribio DVM, PhD. Departments of Veterinary Clinical Sciences. The Ohio State University, Columbus, Ohio 43210

EPIDEMIOLOGY AND APPLIED RESEARCH

EAR - 1

EXTENDED-SPECTRUM CEPHALOSPORIN, CARBAPENEM, AND FLUOROQUINOLONE RESISTANT COLIFORM BACTERIA FROM A LARGE EQUINE TEACHING HOSPITAL AND A REFERRAL EQUINE SPECIALTY HOSPITAL. R. Adams, D. Mathys, D. Mollenkopf, A. Whittle, M. Mudge, A. Bertone, J. Daniels, T. Wittum. Depts. of Veterinary Preventive Medicine and Veterinary Clinical Sciences

EAR - 2

SALMONELLA ENTERICA PREVALENCE IN THE OHIO STATE UNIVERSITY VETERINARY MEDICAL CENTER ENVIRONMENT. A. Albers, D. Mollenkopf, D. Mathys, T. Wittum. Department of Veterinary Preventive Medicine

EAR - 3

INFLUENCE OF A PLANT QUATERNARY BENZO[C]PHENANTHRIDINE ALKALOID-SUPPLEMENTED DIET ON THE INTESTINAL MICROBIOTA PROFILE OF FINISHING PIGS CHALLENGED WITH *SALMONELLA*. V. Artuso-Ponte, S. Moeller, P. Rajala-Schultz, P.N. Boyaka, and W. Gebreyes. Depts. of Veterinary Preventive Medicine, Animal Sciences and Veterinary Biosciences.

EAR - 4

COMPARATIVE HEALTH ANALYSIS OF ENDANGERED MASSASAUGA RATTLESNAKES ACROSS OHIO AND ILLINOIS. K. Backus, M. Freeman, B. Wolfe, G. Lipps, College of Veterinary Medicine.

EAR – 5

COMPARATIVE STUDY OF COMMERCIALY SOLD RAW PET FOOD PROCESSING. P. H. Bellen and T. Wittum. Department of Veterinary Preventive Medicine

EAR - 6

CHARACTERIZATION OF BEHAVIORAL INDICATORS FOR EQUINE PROTOZOAL MYELOENCEPHALITIS (EPM). L. Diangelo, W. Saville, S. Reed, and K. Proudfoot. The Ohio State University Department of Veterinary Preventive Medicine, Columbus, OH (Diangelo, Saville, Proudfoot)

EAR - 7

DISSEMINATION OF ANTIMICROBIAL RESISTANT ENTERIC BACTERIA IN A ZOO ENVIRONMENT. S. M. Feicht, D. A. Mathys, D. F. Mollenkopf, T. E. Wittum, Department of Veterinary Preventive Medicine

EAR - 8

NON-TYPHOIDAL *SALMONELLA* IN VEAL CALVES. S. Finney, L. Munoz, H. Hutchinson, A. Ascot, A. Strait, M. Masterson, G. Habing. Dept. Of Veterinary Preventive Medicine.

EAR - 9

DEVELOPMENT OF MULTILOCUS SEQUENCE TYPING (MLST) ASSAY FOR *MYCOPLASMA IOWAE*. M Ghanem^a and M El-Gazzar ^{a,*}

^aDepartment of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210, USA.

EAR - 10

NON-WOVEN FABRICS FOR NASAL WIPE SAMPLING OF INFLUENZA A VIRUS IN SWINE. CT Hammons, N Bliss, JM Nolting, and AS Bowman. Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio

EAR - 11

CHANGES IN THE PREVALENCE OF ANTIMICROBIAL RESISTANCE THROUGH A VERTICALLY INTEGRATED VEAL CALF PRODUCTION SYSTEM H. Hutchinson, S. Finney, A. Ascott, A. Strait L. Munoz-Vargas and S. Feicht, M. Masterson and G. Habing. Dept. of Veterinary Preventative Medicine.

EAR - 12

ENVIRONMENTAL SURVEILLANCE FOR EXTENDED SPECTRUM β -LACTAMASE GENES IN *ESCHERICHIA COLI* AT A MUNICIPAL WASTEWATER TREATMENT PLANT. CA King, DF Mollenkopf, DA Mathys, DM Stuever, JB Daniels, TE Wittum. Departments of Veterinary Preventive Medicine and Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University.

EAR - 13

COMPARISON OF THE MICROBIOLOGICAL QUALITY OF FRESH PRODUCE FROM SEASONAL FARMER'S MARKETS AND RETAIL GROCERY STORES IN OHIO. D. I. Korec, D. A. Mathys, D. F. Mollenkopf, T. E. Wittum. The Ohio State University, College of Veterinary Medicine, Department of Veterinary Preventive Medicine, Columbus, OH

EAR - 14

DETECTION OF PORCINE HEMAGGLUTININATING ENCEPHALOMYELITIS VIRUS IN EXHIBITION SWINE WITH INFLUENZA-LIKE ILLNESS AT AGRICULTURAL FAIRS IN MICHIGAN IN 2015. J. Lorbach, S. Nelson, M. Zentkovich, J. Nolting, A. Bowman. Department of Veterinary Preventive Medicine, The Ohio State University

EAR - 15

DAIRY CALF PREFERENCE FOR ENRICHMENT ITEMS ADDED TO AN OUTDOOR HUTCH. H. Manning, E. Cosentino, J. Pempek, M. Eastridge, K. Proudfoot. Dept. of Veterinary Preventive Medicine

EAR - 16

PLATFORM PRESENTATION

***ENTEROBACTERIACEAE* PRODUCING EXTENDED SPECTRUM β -LACTAMASES (ESBL) FROM WILD BIRDS IN OHIO.** D.A. Mathys¹, B. A. Mathys², D.F. Mollenkopf¹, J.B. Daniels³, T.E. Wittum¹. ¹Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Ohio State University ²Department of Natural Sciences, Ohio Dominican University ³Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University

EAR - 17

AMYLOIDOSIS IN CHEETAHS (*Acinonyx jubatus*)

K.M. McLean,¹ R.B. Garabed,¹ and B.A. Wolfe. ^{1,2} ¹Dept. of Veterinary Preventive Medicine. ²Morris Animal Foundation

EAR - 18

GENOTYPIC CHARACTERIZATION OF EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANT NONTYPHOIDAL *SALMONELLA* FROM THE NAHMS FEEDLOT 2011 STUDY. D. Mollenkopf¹, D. Mathys¹, D. Dargatz², M. Erdman³, J. Daniels⁴, T. Wittum¹. ¹Dept. of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH. ²USDA, APHIS, VS Centers for Epidemiology and Animal Health, Fort Collins, Colorado, ³Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories, USDA, Ames, IA, ⁴Dept. of Veterinary Clinical Science, College of Veterinary Medicine, The Ohio State University, Columbus, OH

EAR - 19

HOST SPECIES HETEROGENEITY IN THE EPIDEMIOLOGY OF *NESOPORA CANINUM*. K. Moreno-Torres, L. W. Pomeroy, B. Wolfe, W. Saville, M. Moritz and R. Garabed. Depts. of Veterinary Preventive Medicine and Anthropology

EAR - 20

TRANSMISSION OF *SALMONELLA* FROM FARM TO FOOD: THE IMPACT OF CLINICAL OUTBREAKS OF SALMONELLOSIS IN CALVES ON RECOVERY OF *SALMONELLA* FROM LYMPH NODES AT HARVEST. L.M. Muñoz-Vargas¹, S. Finney¹, H. Hutchinson² and G. Habing¹. ¹Dept. of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA. ²Dept. of Animal Sciences, The Ohio State University, Columbus, OH, USA.

EAR - 21

FLOW CYTOMETRIC CHARACTERIZATION OF SIALIC ACID RECEPTORS ON MDCK CELLS MAINTAINED UNDER DIFFERENT MEDIA CONDITIONS AND IMPLICATIONS FOR DETECTION OF INFLUENZA A VIRUS. S. Nelson, I. Davis, A. Bowman. Departments of Veterinary Biosciences and Veterinary Preventive Medicine

EAR - 22

EFFECTS OF POSTPARTUM UTERINE DISEASES ON MILK YIELD, MILK COMPONENTS, AND CULLING IN DAIRY COWS UNDER CERTIFIED ORGANIC MANAGEMENT. J. Piñeiro[‡], M. Maquivar[&], A. Barragan[‡], J. Velez[‡], H. Bothe[‡], and G. Schuenemann[‡]. [‡]*Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA*
[&]*Department of Animal Sciences, Washington State University, Pullman, WA*
[†]*Aurora Organic Farms, Boulder, CO, USA*

EAR - 23

DISTRIBUTION AND DIVERSITY OF *SALMONELLA* IN SHIPMENTS OF HATCHLING POULTRY, UNITED STATES, 2013-2015. A. Sharma¹, M.M. Erdman², L. Muñoz-Vargas¹, R. O'Shaughnessy¹, G.G. Habing¹. (1) The Ohio State University, (2) National Veterinary Services Laboratories, APHIS, USDA

EAR - 24

DETECTION OF *HAMMONDIA HEYDORNI* OOCYSTS IN WILD AND DOMESTIC CANID FECES. D. Sinnott, K. Moreno Torres, B. Wolfe, R. Garabed, and A. E. Marsh. Dept. of Veterinary Preventive Medicine.

EAR - 25

EVALUATION OF RISK FACTORS AND TRANSMISSION PATHWAYS OF SALMONELLA AND ESBL-PRODUCING ORGANISMS FOR DOGS ON OHIO LIVESTOCK FARMS. A. Smith, N. Moran, T. Mills, D. Mollenkopf, D. Mathys, T. Wittum, and J. Stull. Dept. of Veterinary Preventative Medicine.

EAR - 26

ASSESSMENT OF THE CANINE RABIES PROGRAM IN ETHIOPIA: A PROJECT OF RIGHT PARTNERSHIP'S PILOT PROGRAM IN NORTH GONDAR Waibel, S., O'Quin, J., and Gebreyes, W. The Department of Veterinary Preventative Medicine

IMMUNOLOGY AND INFECTIOUS DISEASES

IMID-1

SAMHD1-MEDIATED HIV-1 RESTRICTION IN CELLS DOES NOT INVOLVE RIBONUCLEASE ACTIVITY. JM Antonucci^{1,2}, C St. Gelais¹, S de Silva¹, JS Yount³, C Tang⁴

IMID - 2

REGULATION OF IMMUNOGLOBULIN CLASS SWITCH BY PHARMACOLOGICAL INHIBITORS OF INFLAMMATION AND NEUTROPHIL FUNCTIONS. Z. Attia^{1,2}, H.E.Steiner¹, E.Kim¹, T.L.Martin¹, A.Zaghawa², E.Cormet-Boyaka¹, P.N.Boyaka¹. ¹Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, Columbus, OH; ²Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt. , X Ji⁴, C Shepard⁵, Y Xiong⁴, B Kim⁵, L Wu¹

IMID - 3

RANDOM MUTAGENESIS OF *EHRlichia* SP. HF STRAIN FOR IDENTIFICATION OF VIRULENCE GENES. H. Bekebrede, M. Lin, Y. Rikihisa. Dept. of Veterinary Biosciences

IMID - 4

POLY-LACTIC-CO-GLYCOLIC ACID (PLGA) NANOPARTICLE DELIVERY OF SWINE INFLUENZA VIRUS VACCINE PROVIDES HETEROLOGOUS PROTECTION THROUGH CELL MEDIATED IMMUNITY IN PIGS.

S. Dhakal, J. Hiremath¹, K. Bondra¹, Y. SL¹, B. Shyu¹, K. Oyuang¹, B. Binjawadagi¹, K.I. Kang¹, J. Goodman², B. Narasimhan², C.W. Lee¹, R.J. Gourapura¹; ¹Food Animal Health Research Program, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA, ²Department of Chemical and Biological Engineering, Iowa State University, Ames, IA, USA.

IMID - 5

THE EFFECT OF HYPOTHERMIA ON INFLUX OF MONONUCLEAR CELLS IN THE DIGITAL LAMELLAE OF HORSES WITH OLIGOFRUCTOSE-INDUCED LAMINITIS.

J.D. Godman¹, T.A. Burns¹, C.S. Kelly¹, M. Watts¹, B.S. Leise², E.L. Schroeder¹, A.W. van Eps³, J.K. Belknap¹ 1. The Ohio State University, Columbus OH, 2. Louisiana State University, Baton Rouge, LA, 3. The University of Queensland, Brisbane, Australia

IMID - 6

CX3CR1 IN COTTON RATS IS THE RECEPTOR FOR RESPIRATORY SYNCYTIAL VIRUS AS IT IS IN HUMANS.

G Green¹, S. Johnson², A. Oomens⁴, M. Teng³, M. Peeples², S. Niewiesk¹

¹Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio; ²Center for Vaccines and Immunity, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio; ³Division of Allergy and Immunology, Department of Internal Medicine, Morsani College of Medicine, University of South Florida, Tampa, Florida; ⁴Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, Oklahoma.

IMID - 7

EVALUATION OF THE VIRULENCE OF A PORCINE EPIDEMIC DIARRHEA VIRUS WITH A 197 AMINO ACID-DELETION IN THE SPIKE PROTEIN

Y. Hou, C.M Lin, X. Gao, Z. Lu, X. Liu, Y. Qin, L. J. Saif and Q. Wang
Food Animal Health Research Program, Ohio Agricultural Research and Development Center, College of Food, Agricultural and Environmental Sciences, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, Ohio, USA

IMID - 8

CATHEPSIN K INHIBITION RENDERS EQUINE BONE MARROW NUCLEATED PROGENITOR CELLS HYPO-RESPONSIVE TO LPS AND UNMETHYLATED CPG STIMULATION *IN VITRO*.

H. Hussein¹, P. Boyaka², J. Dulin¹, A. Bertone^{1, 2}. ¹.Dept. of Veterinary Clinical Sciences. ². Dept. of Veterinary Biosciences. College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.

IMID - 9

ANTIMICROBIAL USE AND RESISTANCE IN ZONOTIC BACTERIA RECOVERED FROM NONHUMAN PRIMATES.

J. Kim, The Ohio State University, College of Veterinary Medicine, Department of Veterinary Preventive Medicine, College of Public Health, Columbus, Ohio, United States of America; D. J. Coble, The Ohio State University, College of Veterinary Medicine, Department of Veterinary Preventive Medicine, University of Laboratory Animal Resources, Columbus, Ohio, United States of America; G. W. Salyards, University of California, Davis, California National Primate Research Center, Davis, California, United States of America; W. Rinaldi, Alpha Genesis Incorporated, Yemassee, South Carolina, United States of America; G. Plauche, University of California, Davis, California National Primate Research Center, Davis, California, United States of America; G. H. Habing, The Ohio State University, College of Veterinary Medicine, Department of Veterinary Preventive Medicine, Columbus, Ohio, United States of America

IMID - 10

EPITHELIAL CELL IKK β REGULATES EOSINOPHIL LEVELS IN THE INTESTINE AND SEVERITY OF ALLERGIC RESPONSES TO INGESTED ALLERGENS

E. Kim, M. M. Lember, T. L. Martin, J. C. Rowe, H. E. Steiner, E. Cormet-Boyaka, P. N. Boyaka. Depts. of Veterinary Biosciences

IMID - 11

DEVELOPING A CRYOPRESERVATION METHOD THAT PRESERVES FUNCTION OF CANINE AND FELINE PERIPHERAL BLOOD MONONUCLEAR CELLS.

Y. Lin, R. Vicetti Miguel, N. Quispe Calla, K. Henschel, and T. Cherpes. Depts. of Microbial Infection and Immunity and Obstetrics and Gynecology

IMID - 12

EXPERIMENTAL MODELING OF THE NONSPECIFIC PROTECTIVE EFFECTS WITH MEASLES VIRUS VACCINATION.

S. C. Linn, D. Huey, and S. Niewiesk. Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University

IMID - 13

3'3'-CGAMP INDUCES A BALANCED TH1 AND TH2 CYTOKINE PROFILE FOLLOWING SUBLINGUAL IMMUNIZATION.

T. Martin, E. Kim, J. Jee, H.E. Steiner, and P.N. Boyaka. Dept. of Veterinary Biosciences

IMID - 14

MATERNAL ANTIBODY TRANSFER IN THE COTTON RAT PLACENTA

M. E. Martinez¹, K. M. D. La Perle^{1,2}, S. Niewiesk¹

¹Department of Veterinary Biosciences and the ²Comparative Pathology and Mouse Phenotyping Shared Resource, The Ohio State University, Columbus, Ohio

IMID - 15

EFFECT OF NF- κ B PATHWAY IN INTESTINAL EPITHELIAL CELLS DURING INGESTION OF LOW DOSES OF CADMIUM. J. Rowe, E. Kim, H. Steiner, E. Cormet-Boyaka, and P. Boyaka. Dept. Veterinary Biosciences

IMID - 16

EXAGGERATED PRO-INFLAMMATORY INNATE IMMUNE RESPONSE OF CYSTIC FIBROSIS AIRWAY EPITHELIA CELLS TO H1N1 INFLUENZA A INFECTION. S. YOUNG¹, P. Woods¹, M. Peebles², and I. Davis¹. ¹The Ohio State University College of Veterinary Medicine; ²Nationwide Children's Hospital Medical Center

IMID - 17

MIR-155 IMPACTS T CELL MIGRATION IN ACUTE GRAFT-VERSUS-HOST-DISEASE (AGVHD). N.C. Zitzer¹, P.A. Taylor², A. Nganheu¹, Y.A. Efebera¹, S.M. Devine¹, B.R. Blazar², R. Garzon¹, P. Ranganathan¹. ¹Comprehensive Cancer Center, The Ohio State University, Columbus, OH; ²Blood and Marrow Transplantation, Division of Pediatrics, Department of Medicine, University of Minnesota, Minneapolis, MN

IMID - 18

INHIBITION OF LUNG TISSUE NON-SPECIFIC ALKALINE PHOSPHATASE ATTENUATES INFLUENZA-INDUCED ACUTE LUNG. P. S. Woods^{1,2}, L. Doolittle^{1,2}, and I. C. Davis¹ Department of Veterinary Biosciences¹, The Ohio State University. The Ohio State School of Medicine², The Ohio State University

MOLECULAR AND CELLULAR BIOLOGY

MCB - 1

IDENTIFYING THE ROLE OF NOVEL TAX-1 INTERACTING PROTEIN SNX27 IN HTLV-1 INFECTION. Jacob Al-Saleem^{1,2,3}, Nikoloz Shkriabai^{1,4}, Mamuka Kvaratskhelia^{1,4}, Lee Ratner⁶, and Patrick L. Green^{1,2,3,4}

¹Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA; ²Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA; ³College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA; ⁴Department of Pharmaceutics and Pharmaceutical Chemistry, The Ohio State University, Columbus, OH, USA; ⁵Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University, Columbus, OH, USA; ⁶Division of Oncology, Washington University, St Louis, MO, USA

MCB - 2

SALMON POISONING DISEASE: CANINE IMMUNE RECOGNITION OF NEORICKETTSIA HELMINTHOECA. K. Bachman, M. Lin, Y. Rikihisa. Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University

MCB - 3

IMPROVING EFFICIENCY IN SAUGEYE PRODUCTION USING CRYOPRESERVED MILT. B. Blawut¹, M. Krcmarik¹, B. Wolfe³, M.C. da Silva², R. D. Zweifel⁴, D. Sweet⁴, & S. Hale⁴

1 Ohio State University, College of Veterinary Medicine, Department of Veterinary Preventive Medicine, 1920 Coffee Rd. Columbus, OH 43210

2. Ohio State University, College of Veterinary Medicine, Department of Veterinary Clinical Sciences, 601 Vernon Tharp St. Columbus, Ohio 43210

3 Morris Animal Foundation, 720 S. Colorado Blvd., Suite 174A Denver, CO 80246

4 Ohio Division of Natural Resources, 2045 Morse Rd Service Road, Columbus, OH 43229

MCB - 4

THE EFFECT OF TRYPAN BLUE ON POSTERIOR CAPSULE OPACIFICATION IN AN EX VIVO CANINE MODEL BM Brash, DA Wilkie, AJ Gemensky-Metzler, HL Chandler, Department of Veterinary Clinical Sciences, The Ohio State University

MCB - 5

ANTITUMOR ACTIVITY OF SELECTED CHEMOTHERAPY AGENTS ON A FELINE BRONCHIOLOALVEOLAR LUNG CARCINOMA CELL LINE. D. L. Burroughs, S. Roy, and G. Lorch. Department of Veterinary Clinical Sciences.

MCB - 6

CANINE MODEL OF PROSTATE CANCER AND THE ROLE OF THE GASTRIN-RELEASING PEPTIDE RECEPTOR (GRPR). R. Y. Camiener, S. M. Elshafae, W. P. Dirksen, and T. J. Rosol, Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.

MCB - 7

TOPOGRAPHY INFLUENCES GLIAL AND NEURONAL MIGRATION UNDER INFLUENCE OF LAMININ IN VITRO. J Cronin^{1,2}; C Czeisler, PhD^{1,3}; A Short, PhD^{1,4}; J Winter, PhD^{1,4}; JJ Otero, MD, PhD^{1,3}

¹The Ohio State University, ²College of Veterinary Medicine, ³College of Medicine, ⁴College of Engineering

MCB - 8

ANALYSIS OF FAS-MEDIATED APOPTOSIS IN CANINE CERVICAL SPONDYLOMYELOPATHY. E. Curtis, R.C. da Costa. Department of Veterinary Clinical Sciences

MCB - 9

THE EFFECT OF HYPOTHERMIA ON INFLAMMATORY AND GROWTH FACTOR SIGNALING PATHWAYS IN ACUTE LAMINITIS. K. Dern, M. Watts, A. van Eps, J. Belknap. Dept of Veterinary Clinical Sciences

MCB - 10

TRACKING TRANSCRIPTOME MODIFICATIONS RESPONSIVE TO THE ESTROUS CYCLE IN THE MOUSE UTERUS. A. Diedrich, C Koivisto, G Leone. College of Veterinary Medicine (Diedrich, Koivisto), School of Biological Sciences-Molecular Virology, Immunology and Molecular Genetics, College of Medicine (Leone) The Ohio State University

MCB - 11

THE EFFECT OF HDACI (AR-42) ON CANINE PROSTATE CANCER METASTASIS. S. Elshafae¹, N. Kohart¹, L. Altstadt¹, W. Dirksen¹ and T. Rosol¹. Department of Veterinary Biosciences¹, The Ohio State University, Columbus, OH, USA.

MCB - 12

INSULIN-RELATED GROWTH FACTOR SIGNALING EVENTS IN THE EQUINE LAMINAE USING A MODEL OF EQUINE METABOLIC SYNDROME. O. Hegedus, M. Watts, P. Weber, K. Woltman, J. Belknap. Dept. of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University, Columbus, OH (Hegedus, Watts, Balknap). Dept. of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI (Weber and Woltman).

MCB - 13

DOWNREGULATION OF SAMHD1 EXPRESSION CORRELATES WITH INCREASED MICRORNA-181 LEVELS IN SÉZARY SYNDROME PATIENT CD4+ T-CELLS. R. Kohnken¹, K.M. Kodigepalli¹, A. Mishra^{2,3}, P. Porcu^{2,3,4} and L. Wu^{1,2,5}
¹Center of Retrovirus Research, Department of Veterinary Biosciences; ²Comprehensive Cancer Center; ³Division of Hematology; ⁴Department of Internal Medicine; ⁵Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, 43210, USA

MCB - 14

INDUCIBLE CRE-MEDIATED ABLATION OF E2F7 AND E2F8 IN THE MOUSE SMALL INTESTINE. M. Maglaty¹, M. Cuitino², J. Rakijas², and G. Leone². ¹College of Veterinary Medicine; ²Department of Molecular Virology, Immunology, and Medical Genetics, College of Medicine, The Ohio State University.

MCB – 15 PLATFORM PRESENTATION

PRMT5 IS UPREGULATED IN HTLV-1-MEDIATED T-CELL TRANSFORMATION AND SELECTIVE INHIBITION ALTERS VIRAL GENE EXPRESSION AND INFECTED CELL SURVIVAL. A. Panfil, J. Al-Saleem, C. Howard, J. Mates, J. Kwiek, R. Baiocchi, and P. Green. Depts. of Veterinary Biosciences

MCB - 16

CHARACTERIZATION OF LIVING SYNOVIAL EXTRACELLULAR MATRIX SCAFFOLDS FOR GENE DELIVERY. N. Reisbig, H. Hussein, E. Pinnell, A. Bertone. Department of Veterinary Clinical Sciences. College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.

MCB - 17

MICRORNA AS A HOST DETERMINANT OF SEVERITY IN INFLUENZA A VIRUS INFECTION. L.D. Schermerhorn^{*}, P. Woods^{*}, S.P. Nana-Sinkam[&], I.C. Davis^{*}

^{*}Department of Veterinary Biosciences, The Ohio State College of Veterinary Medicine

[&]Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, The Ohio State University College of Medicine

MCB - 18

WNT SIGNALING IN PROSTATE CANCER GROWTH AND BONE METASTASIS.

W. Supsavhad, W. Dirksen, S. Elshafae, B. Hassan, N. Kohart, L. Altstadt and T. Rosol. Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University

MCB - 19

ROLE OF STAT3 IN PROSTATE INVOLUTION AND CANCER CELL DEATH

KA Zabrecky¹, BW Simons², and EM Schaffer²

¹The Ohio State University CVM, Columbus, OH; ²The Brady Urological Institute, Johns Hopkins University, Baltimore, MD

STRUCTURE/FUNCTION

SF - 1

INTRAVENOUS ADMINISTRATION OF COBALT CHLORIDE IS ASSOCIATED WITH HEMODYNAMIC ALTERATIONS IN HORSES. Burns TA, Dembek K, Kamr A, Dooley B, Dunbar LK, Brewington S, Aarnes TK, Bednarski KS, O'Brien C, Lakritz J, Toribio RE.

CLINICAL RESEARCH

MORPHOMETRIC DIFFERENCES OF COMPUTED TOMOGRAPHIC IMAGES BETWEEN THE CERVICAL VERTEBRAL FORAMEN OF GREAT DANES WITH AND WITHOUT SUBCLINICAL NEUROLOGIC DEFICITS. J. Arbogast, R. C. Da Costa DVM, MS, ACVIM. The Ohio State University, College of Veterinary Medicine, Columbus OH

Cervical spondylomyelopathy (CSM), also known as Wobbler's syndrome, is the most common disease of the cervical spine in giant breed dogs. Great Danes suffer from the osseous form of CSM, which results in severe, absolute vertebral canal stenosis secondary to proliferation of the vertebral arch, articular facets, and/or vertebral pedicles. A previous study conducted by Dr. da Costa revealed that more than 50% (17/32) of clinically normal Great Danes displayed neurological deficits upon further evaluation. Our aim is to compare the vertebral foramen of these subclinical dogs to those that are CSM-affected and those that are normal in order to find the cause of these subclinical neurologic deficits. 10 adult Great Danes were enrolled in this study; each participant was clinically normal in its everyday life, but displayed neurologic deficits during a complete neurologic exam. The morphometry of the cervical vertebrae (C2-C7) of each participant was studied using transverse computed tomographic (CT) images and compared to the morphometry of 10 CSM-affected Great Danes and 10 normal Great Danes all of similar age and gender. Subjectively, there didn't appear to be a drastic difference in the vertebrae of the normal and subclinical dogs. However, morphometric analysis revealed that the area of the caudal vertebrae (C5-C7) was a bit smaller in the subclinical dogs in comparison to the normal. Our results indicate that although there does appear to be a minute difference between the vertebrae of normal and subclinical Great Danes, further statistics are needed to determine if they are relevant.

Keywords: Cervical Spodylomyelopathy, wobblers syndrome, Great Danes, Computed Tomography, morphometry, cervical spine

ASSESSMENT OF BIOMARKERS OF PAIN AND ACTIVITY PATTERNS IN LACTATING DAIRY COWS DIAGNOSED WITH CLINICAL METRITIS. A.A.

Barragan¹, J.J. Piñeiro¹, G. M. Schuenemann¹, P.J. Rajala-Schultz¹, D.E. Sanders², S. Bas¹.

¹Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210, ²Vaca Resources, Urbana, OH 43078

Post-partum uterine diseases such as clinical metritis (MET) are associated with substantial economic losses due to reduced milk yield, delayed time to conception, treatment costs, and increased culling and death rates. Furthermore, MET has been characterized by bovine veterinarians as a painful event and can be regarded as a welfare concern since it can be associated with systemic signs, such as fever, depression, loss of appetite, and visceral pain. The objectives of this study were to: 1) assess circulating concentrations of substance P, and 2) daily activity patterns (i.e. lying and standing time) in lactating dairy cows diagnosed with MET. Lactating dairy cows (n=200) from two commercial dairy herds were enrolled in the present study. Cows diagnosed with MET (n=100) at 7 ± 3 days in milk (DIM) were matched according to parity and DIM to cows without MET (noMET; n=100). On study d 1, MET was diagnosed (using a metrichick device) by the presence of watery, reddish or brownish foul smelling vaginal discharge; blood samples were collected for assessment of circulating concentration of substance P. In addition, on study d 1 activity monitors were placed on the hind leg of cows (MET; n = 56; CON; n = 56) and were kept until study d 7. Cows showing any other signs of disease were not included in the study. Cows with MET had higher ($P < 0.05$) plasma concentration of substance P when compared to noMET cows (MET = 72.44 pg/ml; noMET = 55.73 pg/ml). Furthermore, cows with MET tended ($P = 0.06$) to spend more time lying (635.60 vs. 603.02 min/day) and less time standing (804.08 vs. 837.25 min/day) than noMET cows. These findings provide evidence that biomarkers of pain are increased and activity is affected in cows with MET.

Keywords: Dairy Cattle, Metritis, Substance P, Activity

CLINICAL CHARACTERISTICS OF PRESUMPTIVE OR CONFIRMED FIBROCARILAGINOUS EMBOLIC MYELOPATHY (FCE): A SYSTEMATIC REVIEW OF 393 CASES FROM 1973 TO 2013. K. Bartholomew, S. Moore, K. Stover, and N. Olby. Dept. of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University (Bartholomew and Moore); and the Dept of Clinical Sciences, College of Veterinary Medicine, North Carolina State University (Stover and Olby).

Objective – To summarize data from a large cohort of dogs with a clinical diagnosis of fibrocartilagenous embolic myelopathy (FCE) to provide a clear understanding of the natural history of this common cause of spinal cord injury in dogs.

Design – Meta-analysis

Animals – 322 previously reported cases from the literature and 71 previously unreported cases identified by retrospective medical record review at two veterinary teaching hospitals.

Procedures – Source publications were identified through a PubMed central search as well as references from a recent review article and resources from any publication obtained thereby. Previously unreported cases were identified via computerized medical records search at The Ohio State University and North Carolina State University.

Results and conclusions– A diagnosis of FCE was most common in middle aged large breed dogs (30%), although a significant number of small breed dogs (24%) were also reported. The most common neuro-anatomic localization was a T3-L3 myelopathy (33.1%), although other presentations including multifocal signs were observed. CSF findings were normal in most cases, but some dogs had profound increases in total nucleated cell count and protein concentration. Prognosis for recovery of ambulation was good to excellent with 85% of cases regaining the ability to walk unassisted within 3 weeks. Persistent neurologic deficits were common in patients that recovered ambulation (49.1%). When nociception was absent in the affected limbs at initial presentation rate of recovery was lower (10%); however, this data may be confounded by limited follow up in more severe cases. Future prospective studies should prospectively evaluate prognosis for more severely affected patients.

Keywords: FCE, FCEM, fibrocartilagenous embolism, fibrocartilagenous embolic myelopathy, canine

COMPARISON OF ALBUMIN, COLLOID OSMOTIC PRESSURE, VON WILLEBRAND FACTOR, AND COAGULATION FACTORS IN CANINE CRYOPOOR PLASMA, CRYOPRECIPITATE, AND FRESH FROZEN PLASMA. C. Culler¹, M. Iazbik M¹, J. Guillaumin² ¹The Ohio State University Veterinary Medical Center. ² Department of Veterinary Clinical Sciences, Ohio State University

Objective— To compare albumin and coagulation factors levels and colloid osmotic pressure (COP) of cryoprecipitate (CRYO) and cryopoor plasma (CPP), to that of source fresh frozen plasma (FFP).

Design—Prospective in-vitro study.

Setting— University teaching hospital.

Animals—Ten healthy, non-Greyhound dogs enrolled in an academic teaching hospital blood donor program.

Interventions—Fresh blood was obtained from canine blood donors and was separated into FFP and packed red blood cells. The source FFP was further separated into CRYO and CPP. Albumin, COP, fibrinogen, coagulation factors II, V, VII, VIII, IX, X, and von Willebrand factor (vWf) were assessed for each FFP, CRYO, and CPP. Measured variables were compared between the three products.

Measurements and Main Results— The mean albumin and COP in CPP were significantly higher than in FFP, with 31.7 ± 6 g/L in CPP compared to 28.9 ± 0.5 g/L in FFP ($p < 0.001$) and 14.5 ± 0.65 mmHg in CPP compared 12.73 ± 0.31 mmHg in FFP ($p = 0.03$), respectively. CRYO had significantly higher levels of fibrinogen (median 3.8 g/L, 95% CI 2.79-4.91 g/L), factor VIII (mean $427.0 \pm 95.4\%$), and vWf (mean $504.7 \pm 41.39\%$) as compared to the other products (all p values < 0.05). The levels of vitamin K dependent factors II, VII, and X were similar in CPP compared to FFP, although factor IX was lower in CPP ($p = 0.036$). There was no significant difference in factor II or VII levels between the three products.

Conclusions— The mean albumin and COP were highest in CPP, suggesting that CPP may be an alternative for oncotic support and albumin replacement. CRYO could be used to treat vWf, factor VIII, and factor IX deficiencies. As factors II, VII and X in CPP were similar to FFP, CPP may be an option for replacement of most vitamin K dependent factors.

Keywords: Plasma, transfusion, coagulation factors, oncotic pressure

ANDROGEN AND PREGNANE RESPONSE TO STRESS IN CRITICALLY ILL FOALS. K. Dembek¹, K. Timko¹, L. Johnson¹, B. David², B. Barr³, K. Hart⁴, and R. Toribio¹, ¹Department of Veterinary Clinical Sciences, The Ohio State University, ²Hagyard Equine Medical Institute, ³Rood and Riddle Equine Hospital, ⁴College of Veterinary Medicine, University of Georgia

Neonatal infection (sepsis) remains the main cause of death in foals. The hypothalamic-pituitary-adrenal axis regulates the response to sepsis-associated stress. We have shown that relative adrenal insufficiency (RAI), characterized by an impaired cortisol response to stress, is associated with mortality and severity of disease in foals. Most studies in foals have been focused on cortisol, while other adrenocortical steroids (pregnane, androgen) have not been investigated.

We hypothesized that RAI in critically ill foals will involve multiple adrenocortical layers, resulting in decreased glucocorticoid, mineralocorticoid, androgen and pregnane concentrations, which will be associated with severity of disease and non-survivor. We also proposed that septic foals with RAI will have higher ACTH/steroid ratios than healthy foals.

Foals were classified into 3 categories based on severity of disease (septic, sick non-septic [SNS] and healthy) and likelihood of survival (Group-1: 3-18%; Group-2: 38-62%; Group-3: 82-97%). Blood concentrations of adrenocorticotropin (ACTH) and steroid were determined by immunoassays. RAI was defined based on the ACTH/cortisol ratio in healthy foals.

Septic foals had higher ACTH, cortisol, 17 α -OH-progesterone, progesterone, pregnenolone, and androstenedione concentrations as well as ACTH/cortisol, ACTH/progesterone and ACTH/aldosterone ratios compared to SNS and healthy foals ($P < 0.01$). The prevalence of RAI was 30% in septic and 18% in SNS foals. Foals with dehydroepiandrosterone (DHEAS) of 0.4-5.4 ng/mL were 2.5 times more likely to have RAI than those with DHEAS of 5.5-30.5 ng/mL. Foals in Group 1 had higher ACTH, aldosterone, progesterone, and cortisol concentrations as well as ACTH/cortisol, and ACTH/progesterone ratios than foals in Groups 2 and 3 ($P < 0.01$). The progesterone cutoff value below which survival could be predicted was 23.5 ng/mL with 75% sensitivity and 72% specificity.

Our study demonstrated that adrenocortical response to critical illness involves multiple adrenal steroids in addition to cortisol. DHEAS and progesterone were good predictors of RAI and mortality in hospitalized foals.

Keywords: sepsis, equine neonates, endocrinology, adrenal insufficiency

IMPACT OF HEAT TREATMENT ON PREVALENCE OF DIROFILARIA IMMITIS ANTIGEN DETECTION IN FRANKLIN COUNTY SHELTER DOGS. M. Elser, A.E. Marsh, B. DiGangi, C. Dworkin, J. Stull, and J. O'Quin. Dept. of Veterinary Preventative Medicine and the University of Florida, College of Veterinary Medicine.

Diagnosis and management of canine heartworm disease is a significant concern for animal shelters across the United States. As many as 7% of samples from shelter dogs may falsely test negative for the detection of *Dirofilaria immitis* antigen. This effect, thought to be the result of antigen blocking due to immune complex formation, can be minimized with heat treatment of serum samples prior to antigen detection. This study determined the prevalence of antigen blocking in dogs entering the Franklin County Animal Shelter, summer and autumn of 2015. We included secondarily, risk factors for positive antigen test results in the analysis. Physical examination of the dogs, blood sampling, and any history about the dog was recorded. The blood samples collected from dogs >6 months of age were tested on site for *Dirofilaria immitis* antigen using a commercially-available microwell ELISA both before and after heat treatment. Whole blood was also evaluated for the presence of microfilariae using modified Knott's testing. Our results include the prevalence of false negative antigen test and the detection of the microfilaria, *Acanthocheilonema reconditum*, in several samples. Historical factors, clinical factors, and the degree of microfilaremia are included in the analysis. Of the 162 shelter dogs that were included in the study, six dogs were found to be heartworm antigen positive (3.70%). Four of those six antigen positive dogs tested positive only after heat treatment, making the prevalence of antigen blocking 2.47% in the sample population. This suggests that antigen blockers could represent a significant portion of the heartworm positive dogs in our community, and heat treating samples could be a valuable method of detecting them.

Keywords: Heartworm, Shelter Medicine, *Dirofilaria immitis*

USE OF FOOD TO FACILITATE HANDLING OF DOGS DURING VETERINARY VISITS: EFFECTS ON GASTROINTESTINAL FUNCTION AND CLIENT PERCEPTIONS OF THE VISIT. M. Forman, T. Shreyer, T. Buffington, S. Barrett

The Ohio State University, Department of Veterinary Clinical Sciences

Veterinary visits induce variable combinations of fear and anxiety in many pets. Provision of palatable food (Counterconditioning- CC), which changes animals' perceptions of stimuli from negative (fear) to positive (pleasure), is one low stress patient handling strategy used to reduce negative perceptions and make veterinary visits a more positive experience for both patients and clients. Neither the incidence of gastrointestinal (GI) distress after typical veterinary visits, nor those where food is utilized as a low stress handling tool, have ever been quantified to our knowledge. To investigate these effects, the incidence of GI distress in patients after visiting our Community Practice Service (CP), where food is commonly used to facilitate patient handling was documented. Client perceptions about the value of using food for low stress handling, with their pet was also assessed. Food types utilized, and amounts offered and consumed during appointments were recorded. Historical behavioral and GI health were also recorded to determine if these variables affected the incidence of GI distress following appointments. Clients were first contacted the day after the appointment to determine their pet's response(s). In addition, an anonymous survey was sent to all clients to ask their opinion about the use of food as a low stress handling technique. Quantifying the possible GI effects of providing high value foods as CC during veterinary visits, and a better understanding of client perceptions about their use, will help veterinary professionals gain insight into the value of this approach to increasing patient comfort and client satisfaction.

Keywords: low stress handling, behavior, veterinary visit

FIBROBLAST GROWTH FACTOR-23 IN CANINE CHRONIC KIDNEY DISEASE. L. Harjes, V. Parker, K. Dembek, L. Giovanni, M. Kogika, D. Chew, and R.E. Toribio. Department of Veterinary Clinical Sciences, The Ohio State University

Fibroblast growth factor-23 (FGF-23) is recently discovered phosphaturic hormone (phosphatonin). In humans and cats with chronic kidney disease (CKD), FGF-23 concentrations are associated with disease progression and development of renal secondary hyperparathyroidism (RHPT). An increase in FGF-23 is one of the earliest biomarkers of CKD, often preceding hyperphosphatemia and RHPT. It is an independent risk factor for both progression of CKD in humans and cats. The objectives of this study were to measure plasma FGF-23 concentrations in healthy dogs and dogs with CKD and to determine its association severity of CKD as well as phosphorus and parathyroid hormone (PTH) concentrations.

Thirty-four dogs with CKD and 10 healthy dogs were included. Dogs with CKD were staged according to International Renal Interest Society (IRIS) guidelines. A human-specific FGF-23 ELISA was validated for canine samples, showing linearity and intra- and interassay coefficients of variation <7%. Values are presented as median (range). Plasma FGF-23 concentrations in healthy dogs and dogs with IRIS stages 3 and 4 CKD were 315 pg/mL (211-449), 2,302 (455-24,409) and 7,733 (2,520-41,265), respectively ($P<0.01$).

Plasma FGF-23 concentrations were positively correlated with creatinine ($r=0.86$, $P<0.01$), phosphorus ($r=0.69$, $P<0.01$), and PTH ($r=0.72$, $P<0.01$) concentrations. Nineteen (56%) CKD dogs had an FGF-23 concentration above the upper range of normal dogs. Based on healthy dog phosphorus concentrations, 11 (32%) CKD dogs had elevated phosphorus concentrations. Only eight (24%) CKD dogs had hyperparathyroidism.

Plasma FGF-23 concentrations were measurable in normal dogs and in dogs with various stages of CKD. Plasma FGF-23 concentrations increase in proportion to severity of CKD, as has been reported in other species. This study showed that plasma FGF-23 has clinical value in assessing early canine CKD, has prognostic value, and provides better insight on the pathophysiology of CKD and RHPT in dogs.

Keywords: fibroblast growth-factor 23, FGF-23, chronic kidney disease

LACTOFERRIN REDUCES MORTALITY IN PRE-WEANED CALVES WITH DIARRHEA. K. Harris and G. Habing. Department of Veterinary Preventive Medicine

According to NAHMS, calf diarrhea is the most common illness in young calves, and nearly 8% die as a result. Alternatives to antimicrobials are frequently used to treat calf diarrhea on organic operations, but there is little data confirming their effectiveness. The availability of effective antibiotic alternatives could help improve antimicrobial stewardship and reduce usage in cases of diarrhea. Lactoferrin and garlic extract have antimicrobial properties and have shown positive impacts on growth in preweaned calves. We hypothesized that lactoferrin and garlic extract would decrease mortality, improve weight gain, and decrease disease duration in pre-weaned calves with diarrhea. In total, 633 calves with diarrhea were enrolled in a blinded, randomized field trial. Upon diagnosis of diarrhea (fecal score>3), calves were randomized to 3 consecutive days of oral treatments with garlic extract, lactoferrin, or water (control). Calves were clinically evaluated for up to 10 days following enrollment, and body weight was measured at enrollment and 10 days later. Mortality, culling, and farm treatments were recorded. Lactoferrin significantly ($p < 0.05$) reduced the risk of death and culling in the preweaning period. In total, 7.5% (15/198) of calves in the control group died compared to 3% (8/201) of calves treated with lactoferrin. Lactoferrin was similarly effective in reducing mortality in older calves (11-21days of age), with severe diarrhea (fecal score= 4), without hyperthermia (temperature<103.0) and absence of depression (depression score=1) ($p < 0.05$). Neither garlic nor lactoferrin had a significant effect on disease duration or average weight gain during the 10 day period ($p > 0.1$). These results suggest that treatment with lactoferrin is effective as an alternative to antimicrobials to reduce mortality in calves between 11 and 21 days of age with watery diarrhea in the absence of systemic signs of dehydration or depression. If confirmed with additional research, lactoferrin has the potential to reduce antimicrobial use and improve calf health.

Keywords: calf diarrhea, lactoferrin

COMPUTATIONAL FLUID DYNAMICS USING COMPUTED TOMOGRAPHY TO ASSESS AIRWAY RESISTANCE IN ENGLISH BULLDOGS. E.T. Hostnik¹, B.A. Scansen¹, R.E. Zielinski², and S.N. Ghadiali². ¹Department of Veterinary Clinical Sciences and ²Department of Biomedical Engineering, The Ohio State University, Columbus, OH 43210.

Introduction/Purpose

Obstructive airway disease is common in brachycephalic dogs. Stenotic nares, edematous intranasal turbinates, mucosal swelling, and an elongated, thickened soft palate are sources of airflow resistance. Surgery has traditionally focused on resection of excessive nares and soft palate, without objective measures to validate efficacy.

Methods

Twenty-three non-operated brachycephalic dogs were recruited for this pilot study. A 128 multi-detector computed tomography (MD-CT) scan was performed in all dogs, from rostral nares to diaphragm (SOMATOM Definition Flash; Siemens Healthcare). MD-CT examinations were performed using conscious sedation and without endotracheal intubation.

Raw MD-CT data were imported into ScanIP software (Simpleware, Version 7.0) to render a three-dimensional surface mesh model by automatic segmentation using -1024 to -450 Hounsfield units to isolate the air-filled nasal passage from the nares to the caudal soft palate. Three-dimensional surface models were then imported into COMSOL Multiphysics 5.0 with MATLAB (COMSOL, Inc., Version 5.0.1.276) for computational fluid dynamic modeling and calculation of airway resistance.

Results

The nasal passages were modeled and airway resistance calculated in all dogs. Airway resistance varied widely; mean and SD of 9,859.19 +/- 12,818.53 Pa/L/s. Airway resistance did not correlate with age ($r = 0.344$, $P = 0.126$) or weight ($r = -0.058$, $P = 0.803$). In 19/21 dogs, the rostral third of the nasal passage showed the greatest step-up of airflow resistance.

Discussion/Conclusion

Computational fluid dynamics derived from nasal MD-CT can quantify airway resistance in dogs. This methodology may have utility for objectively studying surgical interventions in canine brachycephalic airway syndrome.

Keywords Computational fluid dynamics, Computed tomography, Brachycephalic airway syndrome, English Bulldog

THE FGF-23/KLOTHO AXIS AND ITS ASSOCIATION WITH PHOSPHORUS, CALCIUM, VITAMIN D, PARATHYROID HORMONE, DISEASE SEVERITY AND OUTCOME IN HOSPITALIZED FOALS. A. Kamr^{1,4}, K.A. Dembek¹, B.E. Hildreth III¹, S.M. Reed², N.M. Slovis³, T.A. Burns¹, A. Zaghawa⁴, R.E. Toribio¹. ¹ Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH; ²Rood and Riddle Equine Hospital, Lexington, KY; ³Hagyard Equine Medical Institute, Lexington, KY; ⁴Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt

Hypocalcemia and hyperphosphatemia, low vitamin D metabolite and elevated parathyroid hormone (PTH) concentrations are frequent in critically ill foals; however, the mechanisms leading to these abnormalities remain unclear. Fibroblast growth factor-23 (FGF-23) is secreted by osteocytes in response to increased $1,25(\text{OH})_2\text{D}_3$, PTH, and phosphorus concentrations. FGF-23 with its co-receptor klotho inhibits PTH synthesis, as well as renal 1α -hydroxylase activity and phosphorus reabsorption. However, information on FGF-23 and klotho in hospitalized foals is lacking. The goal of this study was to investigate the FGF-23/klotho axis and its relationship with phosphorus, calcium, PTH, vitamin D, disease severity and mortality in hospitalized foals. A total of 91 newborn foals ≤ 3 days old divided into hospitalized (n=81; 58 septic, 23 sick non-septic [SNS]) and healthy (n=10) groups were included. Blood samples were collected on admission. Serum FGF-23, klotho, PTH, and vitamin D metabolites were measured by immunoassays. Data were analyzed by non-parametric methods and logistic regression. Serum FGF-23 concentrations were significantly higher while klotho, $25(\text{OH})\text{D}_3$, and $1,25(\text{OH})_2\text{D}_3$ concentrations were lower in septic and SNS compared to healthy foals ($P < 0.05$). Septic foals had higher phosphorus and PTH, and low calcium concentrations than SNS and healthy foals ($P < 0.05$). In hospitalized and septic foals, serum FGF-23 concentrations were associated with phosphorus and PTH ($P < 0.05$). In septic foals, serum klotho concentrations were positively associated with low $1,25(\text{OH})_2\text{D}_3$ concentrations ($r_s = 0.42$; $P = 0.01$). Hospitalized foals with the highest FGF-23 and lowest klotho concentrations were more likely to die (OR= 3.3; 95% CI = 1.1-10.3; OR=3.1; 95% CI=1.1-8.7, respectively). Elevated FGF-23 and reduced klotho concentrations in combination with high phosphorus and PTH concentrations suggest that FGF-23 resistance may be implicated in the pathogenesis of hyperphosphatemia and elevated PTH concentrations in critically ill foals.

Keywords: FGF-23; klotho; phosphorus; PTH; vitamin D; sepsis; mortality; hospitalized foals.

THE EFFECTS OF HYALURONAN ALONE OR IN COMBINATION WITH CHONDROITIN SULFATE AND N- ACETYL-D-GLUCOSAMINE ON LIPOPOLYSACCHARIDE-CHALLENGED EQUINE FIBROBLAST-LIKE SYNOVIAL CELLS. A. Kilborne, H. Hussein, A. Bertone. Department of Veterinary Clinical Sciences. College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.

Lipopolysaccharide (LPS) is an established model for induction of inflammatory and degradation cascades associated with arthritis. While the anti-inflammatory efficacy of hyaluronan (HA) is documented, combination therapeutics may have the potential to provide an additive or synergistic anti-inflammatory effect as well as anti-catabolic effect and warrant investigation. Our hypothesis was that HA with chondroitin and N-acetyl-glucosamine (HA-CS-NAG) would be superior to HA alone in cellular protection and anti-inflammatory profiles produced by LPS challenge. The cellular and inflammatory response of equine synovial cells to 2 hour (hr) LPS challenge (20 ng/mL or 50ng/ml) with pre (24hr) and sustained (24hr) HA or HA-CS-NAG incubation have been investigated using an *in vitro* model. The LPS treatment induced a decrease in cell viability ($P < 0.01$) and loss of characteristic fibroblast-like synovial cell culture morphology including loss of cell attachment, cell contraction and rounding, and cell death that was LPS-concentration dependent ($P < 0.001$). The expression of inflammatory products; prostaglandin E_2 , interleukin-6, matrix metalloproteinase-3 and cyclo-oxygenase 2, were increased in response to LPS challenge ($P < 0.05$). Both HA and HA-CS-NAG protected synovial cells from the negative effects of LPS ($P < 0.001$). HA-CS-NAG treatment had greater anti-inflammatory effect than HA alone ($P < 0.03$). Our work demonstrated that HA and HA-CS-NAG can protect synovial cells and the use of a combination product may have additional clinical advantage.

Keywords: equine, hyaluronan, polyglycan, synovitis, lipopolysaccharide.

ADRENOCORTICAL STEROID RESPONSE TO A HIGH DOSE OF ACTH IN HEALTHY AND CRITICALLY ILL FOALS. J.S. Minuto, K.A. Dembek, R.E. Toribio. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH

Neonatal infections are the leading causes of mortality in newborn foals. In response to sepsis-related stress, the hypothalamic-pituitary-adrenal axis (HPAA) is activated, resulting in increased concentrations of adrenocorticotropin (ACTH) and cortisol adrenocortical steroids. However, preliminary studies from our group suggest that some critically ill foals have abnormally low cortisol concentrations despite elevated ACTH, indicating relative adrenal insufficiency (RAI). Cortisol and other adrenocortical steroids are essential for energy and blood pressure regulation, as well as organ differentiation and function. We hypothesized that the response of multiple adrenocortical steroids to a high ACTH dose (100 µg) will be lower in septic foals compared to healthy foals, and those with lowest steroid concentrations and poor response to ACTH (supporting RAI)_will have more severe disease and be more likely to die. Foals <4 days old were divided into: healthy, sick non-septic (SNS), and septic. Blood samples were collected at admission, then foals received 100 µg of ACTH (cosyntropin, IV). Additional blood samples were collected 30 and 90 minutes post ACTH administration. Concentrations of cortisol, aldosterone, and steroid precursors were determined via immunoassays. Changes in steroid concentration over 30 minutes were calculated.

In septic foals, cortisol, 17β-estradiol, pregnenolone, and 17α-OH-progesterone concentrations were significantly increased at all time points compared to healthy foals (P<0.05). Dehydroepiandrosterone (DHEA) concentrations were not significantly different between groups at any time. However, healthy, SNS, and septic foals showed 1-fold, 2-fold, and 4-fold increases in DHEA concentrations respectively 30 minutes after ACTH stimulation (P<0.05). Cortisol response to ACTH was lower in non-survivors compared to survivors. However, the difference was not statistically different.

RAI involves multiple adrenocortical steroids in addition to glucocorticoids. High DHEA and low cortisol responses to high dose of ACTH are good indicators of RAI and disease severity.

Key Words: Hypothalamic-Pituitary-Adrenal Axis, ACTH Stimulation Test, Relative Adrenal Insufficiency, Cortisol, DHEA, Sepsis, Equine, Neonate, Foal

REGIONAL DIFFERENCE IN THE SPINAL EPENDYMAL LAYER OF NORMAL DOGS A Muir, SA Moore. Department of Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio

The mammalian spinal cord has restricted ability for regeneration and repair after spinal cord injury (SCI). In rodents, a subpopulation of cells in the spinal ependymal layer (SEL) exhibits neural stem cell characteristics and contributes to tissue repair and regeneration. Dogs are an important spontaneous model through which to study SCI, but little is known about the SEL in this species. Rodent studies indicate important regional differences in the SEL, but this has not been studied in the dog. Our aim was to evaluate the normal canine SEL for regional differences that may impact recovery after SCI. We examined 4-5 consecutive tissue sections of cervical, thorax and lumbar spinal cord from normal dogs (n=5) using hematoxylin and eosin (H&E), and immunohistochemistry for GFAP, S100, and ki67. We observed statistically significant regional differences in number of total cells in the SEL ($p < 0.05$) with the highest number of cells located in the lumbar region (152 ± 29) and the lowest number located in the thoracic region (109 ± 24) of the spinal cord. GFAP was expressed intermittently in cells of the SEL, while S100 was expressed almost universally consistent with neural crest cell origin. We did not identify significant regional differences in GFAP or S100 expression.. Minimal Ki-67 expression was noted in all regions. These results form a basis to compare regional responses after SCI.

Keywords: Spinal cord injury, immunohistochemistry, spinal ependymal layer, neural stem cell, dog

EVALUATION OF KINEMATIC MAGNETIC RESONANCE IMAGING IN DOGS WITH OSSEOUS-ASSOCIATED CERVICAL SPONDYLOMYELOPATHY. M. Provencher, A. Habing, S. Moore, L. Cook, G. Phillips, and R. da Costa. Departments of Veterinary Clinical Sciences and the Center for Biostatistics

Osseous-associated cervical spondylomyelopathy (OA-CSM) is a condition characterized by static and dynamic spinal cord compressions. The dynamic component of cervical spondylotic myelopathy in humans (hCSM) is evaluated with kinematic MRI (kMRI). The purpose of this study was to evaluate kMRI in dogs with OA-CSM using a positioning device that allowed controlled flexion and extension of the cervical vertebral column. We hypothesized that kMRI would reveal new compressive lesions not identified with standard positioning.

Ten Great Danes and 2 Doberman pinschers with a cervical myelopathy were prospectively evaluated. All dogs underwent MRI in a neutral position using a 3.0-Tesla magnet. The patients were then placed on a positioning device and the cervical vertebral column was first flexed and then extended. Additional images were acquired. Morphologic and morphometric assessments were performed.

In neutral 4 patients had 1 compression, 4 patients had 2 compressions, 3 patients had 3 compressions, and 1 patient had 4 compressions. In flexion 1 patient had 0 compressions, 4 patients had 1 compression, 4 patients had 2 compressions, 2 patients had 3 compressions, and 1 patient had 4 compressions. In extension 2 patients had 1 compression, 1 patient had 2 compressions, 8 patients had 3 compressions, and 1 patient had 4 compressions. Extension was associated with mild compression at C4-C5 ($p=0.02$) that was not noted in neutral or flexion. There were 11/32 compressions in extension that were not present in neutral. The presence of dorsal compression with extension was significant at C4-C5 ($p=0.01$). In 1 patient, a synovial cyst that was not identified in neutral was noted to cause mild spinal cord compression in extension.

Our results support the use of kMRI in patients with OA-CSM to reveal new compressive sites, dorsal compressions and to enhance visualization of extradural compressive lesions, such as synovial cysts.

Keywords: OA-CSM, Wobbler, flexion, extension, dynamic

CLINICAL STUDY OF ECHOCARDIOGRAPHIC VARIABILITY IN ESTIMATING PULMONARY ARTERY PRESSURE AND PULMONARY VASCULAR RESISTANCE IN DOGS. J. Rhinehart, K. Schober, B. Scansen, J. Bonagura.
Depts. of Veterinary Clinical Sciences

Pulmonary hypertension (PH) is an important clinical entity and is related to clinical symptoms and prognosis. We hypothesized that Doppler echocardiographic (DE) indices of PH and pulmonary vascular resistance (PVR) are influenced by a variety of independent factors leading to clinically important variability of DE estimates of PH and PVR in dogs.

Dogs with naturally acquired tricuspid regurgitation (TR) were studied prospectively. All dogs had degenerative valve disease. Target variables during 4 study periods (dogs imaged in lateral recumbency, dogs standing, after defined exercise (6-minute walk test [6-MWT]), and after sedation [0.25mg/kg butorphanol, IM]), were quantified by two different observers. Heart rate (HR), TR flow velocity (TRFV), systolic pulmonary artery pressure (sPAP), PVR, estimates of right atrial pressure, stroke volume, cardiac output (CO), and 23 other echocardiographic variables were quantified. Statistical methods included repeated-measures ANOVA and linear mixed model analysis. $P < 0.05$ was considered significant.

Thirty-eight dogs of 15 small breeds with varying TRFVs (2.33-5.64 m/s) and PVR (2.3-22.0 WU) were studied. Observer and body position during echocardiography (lateral recumbency vs. standing) did not have a significant effect on the target variables. Heart rate declined after sedation (mean reduction 13 ± 24 bpm; $P < 0.05$) and increased after 6-MWT (mean increase 18 ± 24 bpm; $P < 0.05$). Sedation significantly increased mean TRFV ($P < 0.001$; mean \pm SD; 3.43 ± 0.95 m/s vs. 3.75 ± 0.88 m/s) and sPAP ($P < 0.001$; 57 ± 30 mmHg vs. 64 ± 31 mmHg) but did not significantly affect PVR ($P = 0.38$). Post-sedation, TRFV increased in 78% of dogs (range of increase 0.02-1.2 m/sec; 12 dogs increased > 0.5 m/sec). Six-MWT had no effect on TRFV ($P > 0.05$; 3.47 ± 1.03 m/s). Multivariate analysis found a significant association between TRFV, HR, and right ventricular shortening fraction but CO was not significantly associated with TRFV.

These data document relevant variability of DE estimates of PH with sedation being the most important cause.

Keywords: pulmonary hypertension, pulmonary vascular resistance, echocardiography, six-minute walk test, dogs

BEHAVIORAL EFFECTS OF TRAZODONE ON HOSPITALIZED DOGS.

S. Gilbert-Gregory, M.R. Rice, J. Stull, K. Proudfoot, M. Herron. Depts. of Veterinary Clinical Sciences and Preventative Medicine.

The hospital setting can be frightening and anxiety producing, even petrifying, for many dogs. The increase in stress during hospitalization can lead to decreased immune function, delayed wound healing, gastric ulcerations, and cardiovascular abnormalities in canine patients. These factors can affect the outcome of the patient, in addition to the primary reason for hospitalization. Even short-term stress can be detrimental to their health and any effort in recognizing and alleviating their stress will be beneficial. Quantification of stress in animals can be difficult, thus recognizing body language, or behavior signals, has the advantage of being a less invasive method for monitoring stress. Supplementing dogs with medication during hospitalization may help decrease the stress-related behaviors thus decrease anxiety. Trazodone has been studied to aid with post-surgical cage rest in dogs. Trazodone not only induces calmness in dogs, but also decreases anxiety as a serotonin antagonist. During the study, seventeen behaviors were observed to ascertain stress in hospitalized dogs and four (lip licking, panting, whining, and whale eye) were found to have a significant decrease in expression between first and second observation in those dogs administered trazodone. There was significant differences seen in total anxiety and also in the frenetic and freeze summation behaviors following trazodone administration. The decrease in frenetic and freeze summation behaviors in the treatment group, compared to the control group, also supports trazodone reduces signs of stress in the treated patients. It is important for clinicians and general practitioners to recognize behaviors that can convey stress/anxiety. Trazodone is a safe and effective anxiolytic for hospitalized dogs. It can be used during hospital stays to improve the welfare of these patients and this should be the goal of every practitioner, in addition to treating any medical/surgical condition.

Keywords: trazodone, behavior, stress, anxiety, frenetic, welfare, hospital, dogs

THE EFFECT OF INTRAVENOUS MAGNESIUM SULFATE (MgSO₄) ADMINISTRATION IN THE HORSE. S. Schumacher DVM, A. Bertone DVM, PhD, and R.Toribio DVM, PhD. Departments of Veterinary Clinical Sciences. The Ohio State University, Columbus, Ohio 43210

Magnesium is a highly abused substance in equine competition when used as a calming agent. In racing and show horses, magnesium sulfate (MgSO₄) has become a substitute for training. Magnesium sulfate has been used for the treatment of preeclampsia in women and in stroke patients; however the use of MgSO₄ in equine competition horses is not for therapeutic reasons. The mechanism of action for MgSO₄ administration is thought to be multifactorial through peripheral vasodilation and its antagonism at N-methyl-D-aspartate receptors (NMDR). The subsequent reduction in mean arterial pressure and a reduction in sensitivity to glutamate, an excitatory neurotransmitter, may be the cause of the behavior modifying effects claimed by horsemen. The goal of this study was to document plasma, cerebrospinal fluid (CSF), and urine changes in electrolytes (total magnesium [tMg], ionized magnesium [Mg²⁺], total calcium [tCa], ionized calcium [Ca²⁺], sodium [Na⁺], and potassium [K⁺]) following MgSO₄ administration. Cardiac ventricular function was evaluated by echocardiography.

A lumbosacral catheter was placed for collection of cerebrospinal fluid. Hypermagnesemia was induced by administering 50% MgSO₄ solution (30 grams/horse) intravenously over 5 minutes. Serum tMg and Mg²⁺ concentrations increased 2-3 fold while serum total calcium tCa and Ca²⁺ concentrations decreased by 15-20% from baseline values. Plasma Ca²⁺ to Mg²⁺ ratio [Ca²⁺/Mg²⁺] decreased by 65% within five minutes and remained below baseline values for the duration. Differences were seen in post administration left ventricular fractional shortening as well. This work provides novel information on the interactions between Mg²⁺ with electrolytes, and may serve to improve regulatory measures on the indiscriminate use of magnesium in competing horses.

Keywords: Magnesium, calcium, hypermagnasemia,

**EPIDEMIOLOGY
AND
APPLIED RESEARCH**

EXTENDED-SPECTRUM CEPHALOSPORIN, CARBAPENEM, AND FLUOROQUINOLONE RESISTANT COLIFORM BACTERIA FROM A LARGE EQUINE TEACHING HOSPITAL AND A REFERRAL EQUINE SPECIALTY HOSPITAL. R. Adams, D. Mathys, D. Mollenkopf, A. Whittle, M. Mudge, A. Bertone, J. Daniels, T. Wittum. Depts. of Veterinary Preventive Medicine and Veterinary Clinical Sciences

Objective: The heightened use of broad-spectrum antibiotics in veterinary and human medicine provides selection pressure for dangerous antibiotic resistance genes in bacteria. Surveillance of bacterial resistance to clinically important antimicrobials is necessary to maintain the effectiveness of antimicrobials for critical medical cases. Our objective was to estimate the prevalence of clinically significant resistance genes in equine veterinary hospital environments and from feces of their hospitalized patients.

Methods: Environmental and fecal samples were collected from The Ohio State University Galbreath Equine Center (OSUGEC) and a referral equine hospital in Kentucky from May 2015 through the present. Fecal swabs were obtained from equine patients upon admission, at 48 hours, and post 48 hours. Environmental and fecal samples were enriched and inoculated onto selective media to identify extended-spectrum cephalosporin, carbapenem, and fluroquinolone resistance.

Results: Of the 80 hospitalized horses enrolled, patients were significantly more likely to harbor antimicrobial resistance after 48 hours of hospitalization, with odds ratios of 4.14 ($p<0.0001$) 2.02 ($p=0.034$), and 3.13 ($p<0.0001$) for ceftiofur, cefepime, and ciprofloxacin, respectively. Patients were significantly less likely to harbor antimicrobial resistance if they were hospitalized at OSUGEC, with odds ratios of 0.29 ($p=0.012$), 0.26 ($p=0.006$), and 0.14 ($p<0.0001$) for ceftiofur, cefepime, and ciprofloxacin. From the Kentucky hospital, 52%, 38%, and 34% of the 166 surfaces sampled over 3 visits housed isolates resistant to ceftiofur, cefepime, and ciprofloxacin respectively. Over 3 similar visits, 80%, 47%, and 38% of the 96 surfaces from OSUGEC harbored bacteria resistant to ceftiofur, cefepime, and ciprofloxacin.

Discussion: These results show that hospital environmental surfaces are contaminated with resistant bacteria and can serve as reservoirs for antibiotic resistant bacteria. Additionally, longer hospitalization lead to increased carriage of clinically important antimicrobial resistance genes. Antibiotic stewardship and preventing environmental contamination is essential to protect both animal and public health.

Keywords: antimicrobial resistance, equine, nosocomial

***SALMONELLA ENTERICA* PREVALENCE IN THE OHIO STATE UNIVERSITY VETERINARY MEDICAL CENTER ENVIRONMENT.** A. Albers, D. Mollenkopf, D. Mathys, T. Wittum. Department of Veterinary Preventive Medicine

Salmonella is a harmful, often food-borne pathogen that can cause severe dehydration and diarrhea in humans and animals. In a veterinary teaching hospital, the health risk associated with direct *Salmonella* exposure threatens the safety of patients, staff and students. The objectives of this study are to measure the frequency of *Salmonella* in the OSU-VMC hospital environment, and determine if there are resident *Salmonella* strains which are maintained in the VMC. Samples were aseptically collected with an electrostatic cloth from twenty combined floor drains from the equine and food animal areas of the OSU-VMC between February 16 2015, and November 3, 2015. The samples were added to buffered peptone water, transferred to Rappaport-Vassiliadis broth, and inoculated onto XLT-4 and MacConkey agar. To determine if the bacterial growth was *Salmonella* a polyvalent antisera test was performed and the isolates were inoculated onto TSI slants. Pulsed-field gel electrophoresis was used to determine bacterial relatedness using banding patterns of recovered *Salmonella* isolates. A total of 23 *Salmonella* isolates were recovered from 360 (6.4%) environmental samples with prevalence ranging from 0 to 40% on 18 individual sampling dates. A total of 8.9% of food animal service drain samples and 3.9% of equine service drain samples were positive for *Salmonella*. The PFGE indicates that eleven unique *Salmonella* strains were recovered. A single *Salmonella* strain did not appear to persist within the OSU-VMC environment for an extended period of time. The presence of the same *Salmonella* clones recovered from both equine drains and food animal drains on the same date of sampling indicates the possibility of *Salmonella* being transferred between the two services, in addition to the rest of the hospital.

Keywords: *Salmonella*, environment, veterinary hospital, antibiotic resistance

INFLUENCE OF A PLANT QUATERNARY BENZO[C]PHENANTHRIDINE ALKALOID-SUPPLEMENTED DIET ON THE INTESTINAL MICROBIOTA PROFILE OF FINISHING PIGS CHALLENGED WITH *SALMONELLA*. V. Artuso-Ponte, S. Moeller, P. Rajala-Schultz, P.N. Boyaka, and W. Gebreyes. Depts. of Veterinary Preventive Medicine, Animal Sciences and Veterinary Biosciences.

In this study we evaluated the effect of Quaternary-Benzo[c]phenanthridine Alkaloids (QBA) supplementation on the intestinal microbiome of *Salmonella*-challenged pigs. The influence of the transportation stress was also investigated. Fecal samples collected from a total of 47 pigs from 3 treatment groups (T1: in-feed QBA, $n = 16$; T2: in-feed and water soluble QBA, $n = 15$; CON: control non-supplemented, $n = 16$). Specimens were collected from all pigs 14 days after treatment initiation (day 27) and after transport to the slaughterhouse (day 28). Genomic DNA was extracted and used to amplify the V4 variable region of the 16S *rRNA* gene. The amplicons were sequenced on a MiSeq Illumina® Sequencer. Finally, the SILVA *rRNA* gene Database Project was implemented in mothur to assign the unique sequences to phylotypes at a 0.03 dissimilarity cutoff. Using analyses of alpha diversity, we found significant differences in the microbial communities of pigs receiving QBA in the feed and the drinking water as compared to non-supplemented and in-feed QBA supplemented pigs after two weeks of treatment. This was mainly attributed to the significantly lower relative abundance of the family Succinivibrionaceae among pigs in the T2 as compared to T1 and CON groups. Additionally, the microbial community structure in these pigs tended to be different as compared to non-supplemented and in-feed QBA supplemented pigs as implied by the Analysis of Molecular Variance (AMOVA) findings. However, after the pigs were transported to the slaughterhouse differences in alpha diversity were only detected within non-supplemented pigs. No differences in species richness were found within and between treatment groups at day 27 or day 28. Our results suggest that transportation to the slaughterhouse is a stressful event for pigs that can affect intestinal microbiome and QBA supplementation may be a good strategy to maintain the gastrointestinal tract ecosystem.

Keywords: quaternary benzo[c]phenanthridine alkaloids, stress, intestinal microbiota

COMPARATIVE HEALTH ANALYSIS OF ENDANGERED MASSASAUGA RATTLESNAKES ACROSS OHIO AND ILLINOIS. K. Backus, M. Freeman, B. Wolfe, G. Lipps, College of Veterinary Medicine.

Massasauga rattlesnakes are found throughout parts of the United States and are native to Ohio where they are recognized as endangered. Massasaugas will likely be listed under the federal Endangered Species Act within the next few years due to their declining numbers. Research has shown that there is very little correlation between genetics and the body condition and health of massasaugas, suggesting their environment and stressors play a larger role on their success. Blood, body weight, snout-vent length, and *Ophidiomyces ophidiicola* fungal swabs for culture were collected from 23 Massasaugas in the Northeast Ohio region. We are comparing health parameters; such as neutrophil to lymphocyte ratios, body weights and lengths, and snake fungal disease occurrence from the 23 snakes captured in Northeast Ohio to previously published data from Allender et al. (2013) on populations in Illinois. We are also looking at the snake populations' health status as compared to habitat parameters from the 11 different sites in which snakes were collected. All snakes in our population tested negative for *Ophidiomyces ophidiicola*. Analysis on blood parameters and habitat data are pending but initial comparisons show a much lower Neutrophil:Lymphocyte ratio in the snake populations in Ohio than those published for populations in Illinois, possibly suggesting a lower level of chronic stress and inflammation in the Ohio population. These parameters can serve as a baseline for comparison for Ohio populations of Massasauga rattlesnakes in the future to monitor their health and population status.

Keywords: Massasauga, rattlesnakes, endangered, wildlife, ophidiomyces ophidiicola

COMPARATIVE STUDY OF COMMERCIALY SOLD RAW PET FOOD PROCESSING.

P. H. Bellen and T. Wittum. Department of Veterinary Preventive Medicine

Commercial raw pet food is the fastest growing segment in the pet food industry despite having high risk for food-borne pathogens. *Listeria monocytogenes*, was recently discovered in commercial raw pet foods (Nemser, 2014) prompting numerous product recalls as they pose health risks particularly to the immunocompromised population (Jason Ward Stull, 2015). Conversely, in 2014, sales increased by 64% (\$25M to \$40M) in raw freeze-dried and 32% (\$52M to \$69M) in frozen products.

Products are processed under one of four methodologies: raw freeze-dried, frozen, dehydrated and supplemented with high pressurized processing (HPP). The study tested 89 products to compare efficacy between the processing technologies in preventing *L. monocytogenes*, *Salmonella* and multi resistant strain *E.coli* contamination by directly comparing recovery rates. Each product was stored and prepared following product labels. 4 grams samples in 36mL listeria enrichment broth were incubated at 30°C for 48 hours. Broth samples were inoculated to a modified oxford agar plate, incubated at 35°C for another 24 hours. Any growth was inoculated in motility agar and blood plate and incubated for 24 hours at room temperature. Any positive growth for *L. monocytogenes* were confirmed using matrix-assisted laser desorption ionization (MALDI-TOF) system. Results showed 0 *L. monocytogenes*, 25 *E.coli* (23 from raw frozen; 2 raw frozen with HPP) and 7 *Salmonella* (7 from raw frozen) contaminations. The study is ongoing and more data are being collected. Probable correlation in protein type and contamination risk is also analyzed.

The goal is to produce scientifically based data about differences in processing, if any, in efficacy of eliminating pathogen contamination. This will provide guidance regarding the safety of this emerging pet food niche to guide veterinarians and other healthcare professionals in effectively educating pet advocates of the risks and how to mitigate them via right product choices and strict adherence to safe food handling.

Keywords: Raw Pet Food, *L. monocytogenes*, Commercial raw pet food, Food borne pathogens, Zoonotic diseases from pets, Pet food

CHARACTERIZATION OF BEHAVIORAL INDICATORS FOR EQUINE PROTOZOAL MYELOENCEPHALITIS (EPM). L. Diangelo, W. Saville, S. Reed, and K. Proudfoot. The Ohio State University Department of Veterinary Preventive Medicine, Columbus, OH (Diangelo, Saville, Proudfoot)

Equine protozoal myeloencephalitis (EPM) is a debilitating disease that affects the central nervous system of horses. Measuring behavioral changes associated with EPM may aid in early diagnosis and treatment. EPM often affects primarily one side of the body, thus, we hypothesized that EPM horses show more asymmetric behavior compared to horses with another neurological disease, cervical vertebral stenosis myelopathy (CVM). Patient records from 20 confirmed EPM and 20 CVM cases were collected from an equine veterinary hospital. The records were entered into a computer by an observer blind to disease. Records included a 5-point gait assessment (0 = normal to 5 = recumbent) assessing ataxia, dysmetria, paresis, and spasticity at a walk and trot. Weakness was evaluated with a tail-pull test on each side. Twenty-six records included a complete gait assessment (13 per group) and all 40 included a tail-pull. To estimate the severity of gait deficits, a score was calculated by summing the gait assessment for all 4 limbs. A Wilcoxon Two-Sample Test was used to determine if gait severity differed between EPM and CVM. A Fisher's exact test was used to determine if there was a difference in the probability that EPM horses showed more asymmetric tail-pull weakness compared to horses with CVM. There were no differences in any gait category at a walk ($P>0.05$). However, EPM horses had higher scores of dysmetria ($P=0.03$), and tended to have higher scores for spasticity ($P=0.06$) at a trot. A majority of horses with EPM (70%) had asymmetric weakness in the tail-pull test compared to 25% of CVM horses ($P=0.01$). These findings suggest that EPM horses show more asymmetric behavior and gait deficits compared to horses with CVM, which may aid in our ability to detect and treat these horses earlier.

Keywords: Asymmetric gait, Tail pull, Lameness

DISSEMINATION OF ANTIMICROBIAL RESISTANT ENTERIC BACTERIA IN A ZOO ENVIRONMENT. S. M. Feicht, D. A. Mathys, D. F. Mollenkopf, T. E. Wittum, Department of Veterinary Preventive Medicine

Both antimicrobial resistant bacteria and *Salmonella* can contaminate the environment of public animal exhibits such as zoos, which can pose a potential health hazard to both the visitors and the animal population. The objective of this study is to determine the prevalence *Salmonella* contamination as well as extended-spectrum beta-lactam and fluoroquinolone resistant *Enterobacteriaceae* on surfaces of human and animal areas of a large metropolitan zoo. Individual electrostatic cloths were used on flat surfaces of human and animal contact areas, and then enriched in nutrient broth with 2 µg/ml cefotaxime or 16 µg/ml naladixic acid. Incubated cefotaxime broth was inoculated onto MacConkey agar with 8 µg/ml of ceftiofur, 4 µg/ml of cefepime, or 1 µg/ml of meropenem, to identify the *bla*_{CMY}, *bla*_{CTX-M}, and carbapenemase phenotypes. Naladixic acid broth was inoculated onto MacConkey agar with 2 µg/ml of ciprofloxacin or 16 µg/ml naladixic acid, to identify fluoroquinolone resistant phenotypes. A third cloth was enriched in buffered peptone water and Rappaport-Vassiliadis broth, and subsequently inoculated onto XLT-4 agar for the isolation of *Salmonella*. Phenotypic *bla*_{CMY} isolates were found on 34.9% of surfaces, while *bla*_{CTX-M} isolates were found on 12.7% of surfaces. Naladixic acid resistant isolates were found on 36.7% of surfaces, but ciprofloxacin resistant isolates were found on only 18.1% of sampled surfaces. Meropenem resistant isolates were recovered from 4.8% of surfaces sampled. Recovery of antimicrobial resistant bacteria varied between human and animal contact surfaces, with no consistent pattern observed. *Salmonella* were recovered from only 0.6% of surfaces. These results suggest that the zoo environment harbors coliform bacteria resistant to clinically important antimicrobials, and provides an opportunity for a diverse population of humans and animals to be exposed to bacteria expressing multiple antimicrobial resistant phenotypes.

Keywords: Pathogen, Antimicrobial Resistance, Zoo, Environment

NON-TYPHOIDAL *SALMONELLA* IN VEAL CALVES. S. Finney, L. Munoz, H. Hutchinson, A. Ascot, A. Strait, M. Masterson, G. Habing. Dept. Of Veterinary Preventive Medicine.

Non-typhoidal *Salmonella* (NTS) is one of the most important foodborne pathogens in the U.S., causing over 1,000,000 foodborne illnesses annually. *Salmonella* found in the lymphatic tissue of animals at the slaughterhouse is believed to be a source of foodborne *Salmonella* infections. Previous studies have shown an on-farm prevalence of 3.8% to 16.7%, but have not determined associations between on-farm prevalence and prevalence in lymphatic tissue at slaughter. The purpose of this study was to use a longitudinal observational study and vertically integrated veal production system to test the hypothesis of a positive correlation between the prevalence of *Salmonella* recovered on-farm and at slaughter.

Fecal samples were collected from 9 cohorts of calves on 5 different veal farms in addition to environmental and water samples. Four cohorts of calves were followed to slaughter, where mesenteric and pre-femoral lymph nodes and fecal samples were collected from the same calves sampled on-farm. Associations between on-farm shedding and slaughter recovery of *Salmonella* were tested using Chi-square analysis.

The overall prevalence of *Salmonella* on-farm was 1.07% (4/374), but ranged from 0.0% to 4.8% amongst groups. At slaughter, fecal prevalence was found to be 3.75% (6/160), mesenteric lymph nodes showed 20.9% (33/158) and 0.68% (1/148) in pre-femoral lymph nodes. None of the calves that were shedding on-farm were shedding at slaughter. Overall, there was very little correlation between the presence of *Salmonella* in farm fecal samples, slaughter fecal samples, and mesenteric lymph nodes; however, calves with positive mesenteric lymph nodes tended to be more likely to have positive pre-femoral lymph nodes ($p=0.07$). Mesenteric lymph node recovery demonstrates colonization prior to harvest, but low on-farm recovery suggests either infection during lairage or infrequent shedding following infection. *Salmonella* infection in veal calves infrequently led to colonization in peripheral lymph nodes.

Keywords: *Salmonella*, prevalence, farm, slaughterhouse

DEVELOPMENT OF MULTILOCUS SEQUENCE TYPING (MLST) ASSAY FOR *MYCOPLASMA IOWAE*. M Ghanem^a, and M El-Gazzar ^{a,*}

^a*Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210, USA.*

Mycoplasma iowae (MI) infection is an economically and commercially important disease of turkeys. There are no sequence typing assays available for MI strain identification, the only available molecular tools for this purpose, are DNA fingerprinting assays. In addition to their low reproducibility, fingerprinting assays require isolation of the microorganism in pure culture, which is difficult in avian mycoplasma. Therefore, here, we propose a multilocus sequence typing (MLST) assay as the first sequence-typing assay for identification of MI. Based on the two available MI genomes on GenBank, 26 loci of housekeeping genes were identified and studied in a diverse sample set. Six genes were selected for the newly developed MLST assay. The final sequence analysis of six loci (total of 5019bp) (*dppC*, *ulaA*, *valS*, *rpoC*, *leuS*, *kdpA*) allowed the differentiation of 47 MI samples into 23 unique sequence types. Moreover, when only 4 loci were used to type the same set of samples, they resulted in 20 unique sequence types. Analysis of phylogenetic trees and clonal groups generated by MLST displayed a high degree of agreement with geographical and temporal information of the tested samples. MLST results were compared to those of RAPD (Random Amplified Polymorphic DNA), a commonly used DNA fingerprinting assay for avian mycoplasma. MLST results was more consistent than RAPD with epidemiological information. MLST is a highly reproducible molecular epidemiology assay that can be used to identify positive clinical cases directly from DNA samples. Therefore, it provides a useful tool allowing for better identification, control and eradication efforts.

Key words: MLST; *Mycoplasma iowae*; turkeys; Molecular typing

Abbreviations: MLST, multi locus sequence typing; ST, sequence type; RAPD, random amplified polymorphic DNA

NON-WOVEN FABRICS FOR NASAL WIPE SAMPLING OF INFLUENZA A VIRUS IN SWINE. CT Hammons, N Bliss, JM Nolting, and AS Bowman. Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio

Influenza A virus (IAV) is a pathogen with detrimental effects to human and animal health by causing disease in a variety of host species. Pigs can serve as mixing vessels for IAV reassortment and contact between swine and human populations can result in bi-directional zoonotic IAV transmission. Therefore, it is important to optimize IAV surveillance methods in swine populations to monitor the rapid, ongoing IAV evolution occurring in pigs. Cotton gauze is currently used for non-invasive nasal wipe sampling in swine and the objective of this study was to improve the method by investigating the molecular detection and viable IAV recovery from six alternative wipe materials, a variety of non-woven polyester fabrics. Three 25.08 cm² swatches of each fabric were inoculated with IAV (1.0×10^7 TCID₅₀/swatch), placed in vials containing 5 ml viral transport media, and frozen at -80°C until testing was initiated. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to measure molecular detection of virus, and viable IAV recovered from each sample was quantified in cultured MDCK cells. Pairwise comparison between fabrics (TCID₅₀ and number of target copies) was performed using Mann-Whitney rank sum. While none of the fabrics yielded significantly more IAV copies using qRT-PCR, significantly more viable IAV was recovered from fabrics A, B, and C than cotton ($p=0.05$). Fabric A yielded significantly more IAV than both B and C ($p=0.05$). Many factors may play a role in fabric efficacy for virus collection, including fabric composition and absorbency. The present study demonstrates that there are alternatives to cotton gauze that can improve nasal wipe sampling methods for IAV detection in pigs.

Keywords: influenza; IAV; swine influenza; nasal wipe sampling; fabric comparison; influenza surveillance

CHANGES IN THE PREVALENCE OF ANTIMICROBIAL RESISTANCE THROUGH A VERTICALLY INTEGRATED VEAL CALF PRODUCTION SYSTEM H. Hutchinson, S. Finney, A. Ascott, A. Strait L. Munoz-Vargas and S. Feicht, M. Masterson and G. Habing. Dept. of Veterinary Preventative Medicine.

Antimicrobial resistance is a public health concern for both human and veterinary medicine. In food animal production systems, medically important antimicrobials are used for both prophylactic and therapeutic purposes; therefore, food animals have the potential to serve as a reservoir for antimicrobial resistant bacteria. Previous research has shown an uneven distribution of resistance with a higher prevalence within young animals; however, limited research has addressed antimicrobial resistance within veal production systems. Vertically integrated veal production systems provide a unique opportunity to study the transmission of resistance through the food supply. The study's objective was to estimate the prevalence of antimicrobial resistant *Escherichia coli* within different stages of a vertically integrated veal production system. A total of 377 fecal samples were collected from nine different calf cohorts on six farms, where the average age was 69 days (range: 8-115). Four of these cohorts were followed to harvest for additional sample collection. At harvest, a total of 159 fecal samples, 161 pre-evisceration and 150 post-evisceration carcass swabs were collected. A single *E. coli* isolate from the samples was subjected to twelve antimicrobials using Kirby-Bauer disk diffusion assays. Zones of growth inhibition were measured for each antimicrobial and determined resistant based on CLSI standards. Isolates were obtained from 100% of fecal samples, 52% (84/161) of pre-evisceration swabs and 16% (24/150) of post-evisceration swabs. Greater than 98% (372/377) of isolates obtained from farm fecal samples were resistant to two or more antimicrobials. A decrease in resistance was seen at harvest where only 46.9% (73/159), 69.0% (58/84), and 29.2% (7/24) of isolates from fecal samples, pre-evisceration and post-evisceration carcass swabs, were resistant to two or more antimicrobials. These results provide insight to the current prevalence of resistance among the production system and the opportunity for further research to determine factors affecting the prevalence of resistance.

Key Words: *Escherichia coli*, antimicrobials, resistance, veal

ENVIRONMENTAL SURVEILLANCE FOR EXTENDED SPECTRUM β -LACTAMASE GENES IN *ESCHERICHIA COLI* AT A MUNICIPAL WASTEWATER TREATMENT PLANT. CA King, DF Mollenkopf, DA Mathys, DM Stuever, JB Daniels, TE Wittum. Departments of Veterinary Preventive Medicine and Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University.

In response to ever increasing use of antibiotics, bacteria are evolving resistance to critical frontline antimicrobial drugs that treat invasive Gram-negative infections. The most serious threat is bacteria that are resistant to carbapenem drugs. Bacteria may gain this resistance by acquiring mobile resistance genes that confer the ability to produce enzymes that inactivate the antibiotic. Numerous genes, including the *bla*_{KPC} and *bla*_{NDM-1} are known to encode bacteria the ability to produce carbapenemase. While both are present in the US, *bla*_{KPC} has emerged and disseminated primarily in the US, while *bla*_{NDM-1} has primarily disseminated in SE Asia. Because of the frequency of international travel we hypothesized that both *bla*_{KPC} and *bla*_{NDM-1} could be present in Ohio waste-water treatment plants. The purpose of this study was to determine if carbapenem-resistant *E. coli* were present in Columbus wastewater, and to fully characterize those isolates and their resistance mechanisms. We collected 334 samples of untreated sewage water at the Jackson Pike Wastewater Plant between June and August of 2011 and 2012. Using selective media, we identified 158 (47.3%) samples with suspect colonies that grew in the presence of 1 mg/L of meropenem. Of these, 51 (32.9%) were classified as meropenem resistant using Kirby-Bauer disk diffusion assay and 29 isolates were also confirmed to be *E. coli* using biochemical tests and PCR. These isolates were resistant to most of the 26 drugs on our MIC panels using micro-broth dilution. Carbapenemase production was verified for 76 isolates using the Modified Hodge test. However, none of the isolates were positive on the EDTA Double Disk Diffusion test, indicating absence of metallo- β -lactamase production. Our detection of these isolates suggests the presence of a reservoir of important resistance genes for pathogens. Surveillance is an important component of education, awareness, and prevention of antimicrobial resistance in the public health sector.

Keywords: Antibiotic resistance, carbapenems, β -lactamase, *E. coli*

COMPARISON OF THE MICROBIOLOGICAL QUALITY OF FRESH PRODUCE FROM SEASONAL FARMER'S MARKETS AND RETAIL GROCERY STORES IN OHIO. D. I. Korec, D. A. Mathys, D. F. Mollenkopf, T. E. Wittum. The Ohio State University, College of Veterinary Medicine, Department of Veterinary Preventive Medicine, Columbus, OH

The frequent use of antimicrobial drugs in veterinary medicine can result in the emergence and dissemination of antimicrobial resistance in a variety of animal populations. β -lactamases confer bacterial resistance to critically important antimicrobial drugs used in both human and veterinary medicine. Livestock are an important emergence reservoir for zoonotic food-borne transmission of resistant enteric bacteria including *Salmonella* spp. Our aim is to describe the role of fresh produce, which may have been fertilized with livestock feces, in the zoonotic food-borne transmission of antimicrobial resistant bacteria. Samples of leafy greens, tomatoes, and cucumbers were purchased each week from various local farmer's markets and grocery stores. These samples were placed in buffered peptone water (BPW) and inoculated onto spread plates for detection and quantification of coliform bacteria. An aliquot of the BPW was cultured for the presence of *Salmonella*. To test for the presence of β -lactamase-producing bacteria, samples were enriched in a nutrient broth 2 μ g/ml cefotaxime, then inoculated onto 3 MacConkey agar containing Cefoxitin, Cefepime, or Meropenem. We sampled 93 farmer's markets and 67 grocery stores. There are 6 samples which produced isolates resistant to cefoxitin and cefotaxime antimicrobials, indicating the *bla*_{CMY} phenotype. No cefepime or carbapenem resistant isolates were recovered. The mean coliform count was 27 and 22 CFU per 100 μ l BPW rinsate for farmer's markets and grocery stores, respectively. No *Salmonella* spp. were detected. Our results indicate that there is little difference in microbiological quality between farmer's market and grocery store produce measured by the presence of antimicrobial resistant enteric bacteria or coliform contamination.

Keywords: antimicrobial, resistance, lactamases, enteric, *Salmonella*, greens, coliform, bacteria, markets, grocery, microbiological, veterinary

DETECTION OF PORCINE HEMAGGLUTINATING ENCEPHALOMYELITIS VIRUS IN EXHIBITION SWINE WITH INFLUENZA-LIKE ILLNESS AT AGRICULTURAL FAIRS IN MICHIGAN IN 2015. J. Lorbach, S. Nelson, M. Zentkovich, J. Nolting, A. Bowman. Department of Veterinary Preventive Medicine, The Ohio State University

Swine exhibitions at agricultural fairs represent a swine-human interface and necessitate active surveillance for zoonotic agents like influenza A virus (IAV). During routine IAV surveillance activities in the summer of 2015, 6 fairs in Michigan noted acute outbreaks of influenza-like illness (ILI) in exhibition swine. Samples from pigs at these sites tested negative for typical swine respiratory pathogens including IAV. Additional diagnostics detected porcine hemagglutinating encephalomyelitis virus (PHEV) in the samples. Subsequent testing of all 14 Michigan fairs participating in IAV surveillance detected PHEV in 108 of 279 individual samples (38.7%) and at 10 of the 14 fairs (71.4%). Twenty-eight selected Ohio and Indiana fairs participating in IAV surveillance during the summer of 2015 were chosen on the basis of clinical signs and IAV-negative status to serve as controls in a case-control study of the PHEV outbreak in Michigan fairs. PHEV was detected in 23 of 560 control samples (4.1%) and at 4 of 28 control fairs (14.3%). The detection of PHEV was strongly associated with samples from Michigan fairs compared to Ohio and Indiana fairs (OR 14.7; 95% CI, 9.1 to 23.9). PHEV is a known enzootic pathogen of many swine herds, classically causing wasting, vomiting, and encephalitis among piglets. Given these findings typically associated with PHEV infection, the ILI in swine in this study is considered an uncommon presentation. The association of PHEV in exhibition swine with clinical respiratory signs not attributable to typical respiratory agents indicates the virus could play a role in respiratory disease in market age swine. Whether this reflects an under described presentation of PHEV in naïve swine populations, an atypical form of disease, or increased virulence of a particular strain of virus remains unclear. Additionally, this particular outbreak highlights an undescribed exhibition swine network in Michigan that appears segregated from Ohio and Indiana.

Keywords: Influenza A virus, orthomyxovirus, Porcine hemagglutinating encephalomyelitis virus, coronavirus, swine, agricultural fair

DAIRY CALF PREFERENCE FOR ENRICHMENT ITEMS ADDED TO AN OUTDOOR HUTCH. H. Manning, E. Cosentino, J. Pempek, M. Eastridge, K. Proudfoot. Dept. of Veterinary Preventive Medicine

Housing pre-weaned dairy calves in individual pens or hutches is commonplace; however, this housing restricts social contact, and may hinder the behavioral repertoire of calves. This study aimed to determine if calves would use enrichment items if they were added to the hutch, and what type of enrichment they prefer. Ten Jersey heifer calves were housed in individual hutches. The outdoor enclosure of each hutch (1.2m x 2.7m) contained: two artificial teats (1 perpendicular, 1 a 45-degree angle), a stationary brush (L-shaped, two 46cm push brooms), a calf 'lollie' (60cm x 7.2cm PVC pipe with 9.5mm holes for the throughput of dried molasses), and a rubber chain link (30.5cm). The location of each item was alternated per hutch. Video recordings, taken twice weekly during wk 1, 3, and 5 of age from 0800 to 2000h, were used to determine calf preference and use of the enrichment items. Behavior data were not normal, so each variable was log transformed before analysis. A t-test was used to compare the frequency at which calves used each item (averaged across periods), and a repeated measures ANOVA was used to determine if enrichment use changed over time (SAS, Version 9.4). Preliminary analysis revealed no difference between usage of the two teats; thus, these variables were combined. Calves used the brush most frequently, followed by the lollie, chain, and teats (mean \pm SE: 16.9 \pm 1.2; 13.0 \pm 1.2; 8.9 \pm 1.2; 4.7 \pm 1.2 no./12h, respectively; $P < 0.01$). Brush and lollie use increased as calves aged (brush: 10.0 to 25.8 \pm 1.3 no./12h, $P < 0.05$; lollie: 7.6 to 18.5 \pm 1.3 no./12h, $P < 0.05$; wk 1 to 5), but chain and teat use remained similar over time. Results indicate that calves preferred the brush to the other enrichment items. Further analysis is needed to determine the impact of enrichment on positive behaviors (e.g., play) and abnormal behaviors (e.g., non-nutritive or cross-sucking).

Keywords: brush, pre-weaning calf, environmental enrichment

PLATFORM PRESENTATION

ENTEROBACTERIACEAE PRODUCING EXTENDED SPECTRUM β -LACTAMASES (ESBL) FROM WILD BIRDS IN OHIO. D.A. Mathys¹, B. A. Mathys², D.F. Mollenkopf¹, J.B. Daniels³, T.E. Wittum¹.

¹Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Ohio State University ²Department of Natural Sciences, Ohio Dominican University ³Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University

ESBLs confer bacterial resistance to critically important antimicrobials. Livestock are an important reservoir for the zoonotic food-borne transmission of resistant enteric bacteria. Our aim is to describe the potential role of migratory and resident wild birds in the epidemiology of ESBL mediated bacterial resistance on dairy farms. Using mist nets, we sampled wild migratory and resident birds either immediately adjacent to or 600 feet away from free stall barns on three Ohio dairy farms during 2014/2015 spring migration. Individual swabs were used to obtain both a cloacal and external surface swab from each bird. Additionally, wild ducks were sampled either live caught or hunter harvested from hunting preserves in 2014 and 2015. Samples were inoculated into MacConkey broth containing cefotaxime and inoculated onto MacConkey Agar with ceftiofur, cefepime, or meropenem to identify the *bla*_{CMY}, *bla*_{CTX-M}, and carbapenemase phenotypes, respectively. Six hundred and six birds were sampled, 14 (2.3%) of which harbored bacteria with the *bla*_{CMY} gene and 26 (4.3%) harbored bacteria with the *bla*_{CTX-M} gene from either their cloacal sample or from their external swab. There was no difference in the prevalence of either gene between migratory and resident birds. Prevalence of the *bla*_{CMY} was higher among birds sampled immediately outside the barns compared to those sampled 600 feet away. Six hundred and twenty seven ducks were sampled, with 44 (7%) harboring *bla*_{CMY} bacteria and 2 (0.3%) harboring *bla*_{CTX-M} bacteria. Our results suggest that wild birds can serve as mechanical and/or biological vectors for *Enterobacteriaceae* with resistance to extended spectrum cephalosporins. Birds live in close contact with dairy cows and their feed, therefore transmission locally from farm to farm is possible. Finding a similar prevalence in migratory and non-migratory birds suggests the potential for regional and intercontinental movement of these genes via birds.

Keywords: Antibiotic resistance, wildlife, vector, livestock

AMYLOIDOSIS IN CHEETAHS (*Acinonyx jubatus*)

K.M. McLean,¹ R.B. Garabed,¹ and B.A. Wolfe.^{1,2} ¹Dept. of Veterinary Preventive Medicine. ²Morris Animal Foundation

Amyloidosis is a chronic, protein misfolding disorder that causes pathology through the accumulation of misfolded amyloid A protein in visceral organs, often leading to death of the animal. The continued increase of amyloidosis in captive cheetahs (*Acinonyx jubatus*) is of grave concern for the species, yet nothing is definitively known about its mechanism of transmission. Several hypotheses have been presented suggesting varying modes of transmission, including infectious, genetic, or catalyst-dependent transmission. To compare all hypotheses, an agent-based disease model was designed, then populated with demographic and past captive transfer data collected from the 2013 cheetah studbook. Simulation outputs were then compared to historical amyloidosis infection data supplied by the cheetah species survival plan pathologist. Our analysis does not disprove any one hypothesized route of transmission, but rather suggests a multi-faceted route of transmission. Only a subset of the captive population's post mortem disease data were available for this study, and given that the results contradict previous reports, a broader population survey should be pursued.

Keywords: *Acinonyx jubatus*, amyloidosis, cheetah, metapopulation, odds ratio, agent-based disease model

GENOTYPIC CHARACTERIZATION OF EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANT NONTYPHOIDAL *SALMONELLA* FROM THE NAHMS FEEDLOT 2011 STUDY.

D. Mollenkopf¹, D. Mathys¹, D. Dargatz², M. Erdman³, J. Daniels⁴, T. Wittum¹. ¹Dept. of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH. ²USDA, APHIS, VS Centers for Epidemiology and Animal Health, Fort Collins, Colorado, ³Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories, USDA, Ames, IA, ⁴Dept. of Veterinary Clinical Science, College of Veterinary Medicine, The Ohio State University, Columbus, OH

In the US, nontyphoidal *Salmonella* are a common foodborne zoonotic gastroenteritis pathogen. Invasive *Salmonella* infections caused by extended-spectrum cephalosporin resistant (ESCR) phenotypes are more likely to result in treatment failure and adverse health outcomes, especially in severe pediatric *Salmonella* infections where the extended-spectrum β -lactams are the therapy of choice.

To examine the genetic characteristics of ESCR *Salmonella* which may enter the food chain, we characterized 44 ceftiofur-resistant *Salmonella* isolates from the National Animal Health Monitoring System (NAHMS) 2011 beef cattle feedlot health and management project.

As part of the NAHMS Feedlot study, 5,050 individual fecal samples from 68 large (1,000+ head capacity) feedlots were cultured for *Salmonella* spp. The resulting 460 positive samples yielded 571 *Salmonella* isolates with 111 samples (24%) having multiple serotypes. The most prevalent serotypes were *S. Anatum* (n=103, 18%), *S. Montevideo* (n=98, 17%), and *S. Kentucky* (n=87, 15%).

Of the 571 feedlot *Salmonella* isolates, 44 (8%) expressed an AmpC β -lactamase phenotype. These phenotypic *bla*_{CMY} *Salmonella* isolates represented 8 serotypes, most commonly *S. Newport* (n=14, 32%), *S. Typhimurium* (n=13, 30%), and *S. Reading* (n=5, 11%), followed by *S. Dublin*, *S. Infantis*, *S. Montevideo*, *S. Rough O:i:v:1;7*, and *S. Uganda*.

Carriage of the *bla*_{CMY} gene was confirmed for all isolates by PCR. Additionally, all 44 isolates were PCR-positive for the presence of an Inc A/C plasmid which has been previously reported to harbor *bla*_{CMY} in multiple species. Other plasmids, including Inc N, FIC, and FIIA, were also detected in some isolates.

Most *Salmonella* infections are the result of zoonotic foodborne transmission from livestock reservoirs where extended-spectrum cephalosporins are commonly used. Our characterization of the NAHMS Feedlot Surveillance ESCR *Salmonella* shows that while other cephalosporin resistance mechanisms have been reported in US cattle, specific serotypes harboring *bla*_{CMY} on Inc A/C plasmids may be the dominant resistance genotype.

Keywords: *Salmonella*, extended-spectrum cephalosporin resistance, beef feedlot

HOST SPECIES HETEROGENEITY IN THE EPIDEMIOLOGY OF *NESOPORA CANINUM*. K. Moreno-Torres, L. W. Pomeroy, B. Wolfe, W. Saville, M. Moritz and R. Garabed. Depts. of Veterinary Preventive Medicine and Anthropology

The role of host species heterogeneity in the epidemiology of Neosporosis remains under studied, although it is clear that a number of herbivores species are susceptible to *Neospora caninum* infection. Our goal was to better understand the role of host species heterogeneity in the epidemiology of *N. caninum* circulating in a community. We determined immunological and transmission dynamics by comparing catalytic and reverse catalytic infectious disease models with age-structured and constant force of infection in three co-located ruminant populations. Also, we estimated the species-specific contribution to the persistence of this pathogen in the community by calculating the reproductive number of each population. Finally, we calculated the critical vaccination coverage to prevent an outbreak. Results show that immunity in cattle and Pere David's deer wanes over time, suggesting that boosting immunity with vaccines might be a venue to prevent infection within those populations. For white-tailed deer, immunity is lifelong, thus natural boosting of the immune system might be occurring. Cattle's reproductive number was below threshold ($R_t < 1$), meaning that transmission cannot be maintained within cattle, thus an outside source is needed to re-introduce the pathogen and highlighting the importance of controlling outside sources. Pere David's deer and white-tailed deer, both can maintain continues chains of transmission ($R_t > 1$) within their populations. Therefore, control of outside sources might not do a difference. Understanding the epidemiology of multi-host pathogens at the community level allow us to better evaluate processes and transmission dynamic heterogeneities, that could ultimately guide targeted control and with further evaluation the confirmation of reservoirs.

Keywords: catalytic model, community, ruminant, heterogeneity, reservoirs, targeted control, multi-host parasite

TRANSMISSION OF *SALMONELLA* FROM FARM TO FOOD: THE IMPACT OF CLINICAL OUTBREAKS OF SALMONELLOSIS IN CALVES ON RECOVERY OF *SALMONELLA* FROM LYMPH NODES AT HARVEST. L.M. Muñoz-Vargas¹, S. Finney¹, H. Hutchinson² and G. Habing¹. ¹Dept. of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA. ²Dept. of Animal Sciences, The Ohio State University, Columbus, OH, USA.

Cattle are an important reservoir of Non-typhoidal *Salmonella* (NTS) which can be transmitted to humans by consumption of contaminated meat. *Salmonella* can colonize peripheral lymphatic tissues of infected animals that can later be ground with muscles and adipose tissue into ground beef. Outbreaks of salmonellosis occur frequently in cattle on dairy farms, and may result in a higher probability of foodborne transmission of pathogenic strains of NTS. The aims of this study were to 1) compare the prevalence of NTS of cohorts of veal calves with or without a documented recent outbreak of salmonellosis, and 2) assess the genetic relatedness of NTS isolated at farm and slaughter level to evaluate the farm to food transmission. We hypothesized that 1) farms having a recent salmonellosis outbreak would have higher prevalence of NTS in lymph nodes than farms with non-outbreak, and that 2) subtypes found on farm would be closely related genetically to those isolated from lymph nodes. Fecal swabs, mesenteric and pre-femoral lymph nodes of 242 calves from 6 farms (two outbreak and four non-outbreak herds) were collected at harvest. All samples were cultured for *Salmonella* isolation, and 76 isolates were characterized by pulsed-field gel electrophoresis (Xba1-PFGE). Prevalence of *Salmonella* was significantly different ($p \leq 0.05$) between outbreak and non-outbreak farms in feces, mesenteric and pre-femoral lymph nodes, with 11.25%(9/80), 1.25%(1/80), 6.25%(5/80), and 4.3%(7/162), 19.7%(32/162), and 0.6%(1/162), respectively. Indistinguishable PFGE subtypes were recovered from samples between clinically infected farms. These findings suggest that transmission of NTS strains can occur from veal farms to food through lymphatic tissues, and that a highly pathogenic strain has been propagated between some veal farms causing clinical infections. These data demonstrate that implementation of pre-harvest biosecurity measures should be highly recommended in order to decrease the prevalence of NTS in farms, and to prevent the meat contamination at harvesting.

Keywords: *Salmonella*, lymph nodes, veal, calves, PFGE

FLOW CYTOMETRIC CHARACTERIZATION OF SIALIC ACID RECEPTORS ON MDCK CELLS MAINTAINED UNDER DIFFERENT MEDIA CONDITIONS AND IMPLICATIONS FOR DETECTION OF INFLUENZA A VIRUS. S. Nelson,

I. Davis, A. Bowman. Departments of Veterinary Biosciences and Veterinary Preventive Medicine

Influenza A virus (IAV) initiates infections by binding to host-cell surface receptors containing sialic acid. Avian-lineage IAVs preferentially bind α -2,3-linked sialic acids while human-lineage IAVs prefer α -2,6-linked sialic acid receptors. Historically, Madin Darby Canine Kidney (MDCK) cells have been used to isolate IAVs from many species because these cells express both α -2,3-linked and α -2,6-linked receptors. Our hypothesis was that cell culture mediums would alter the relative proportions of α -2,3-linked and α -2,6-linked sialic acid receptors on MDCK cells. Cells were cultured in either serum free media (SFM) or medium containing fetal bovine serum (FBS). Cells from each treatment were stained with sialic acid residue specific dyes, which were subsequently detected using flow cytometry. Cells cultured in SFM consistently expressed both sialic acids whereas cells cultured with FBS had varying proportions that alternated passage by passage. To confirm the biological significance of these differences, 50% tissue culture infectious dose experiments were performed at two successive passage points. Serial dilutions were made of one swine origin IAV and one avian origin IAV and each dilution was inoculated into 8 wells of three 96 well tissue culture plates per media group. The swine IAV grew to similar titers in both culture mediums, while the avian IAV grew to significantly ($p=.014$ for trial 1 and $p=.003$ for trial 2) higher titers in cells maintained in SFM. The cells maintained in SFM were shown to be expressing more α -2,6-linked sialic acids while the cells maintained with FBS were expressing mostly α -2,3-linked sialic acids. The results indicate that culture media can influence the sialic acid expression of MDCK cells and this can alter the efficiency of IAV isolation.

Keywords: MDCK, cell culture, flow cytometry, influenza

EFFECTS OF POSTPARTUM UTERINE DISEASES ON MILK YIELD, MILK COMPONENTS, AND CULLING IN DAIRY COWS UNDER CERTIFIED ORGANIC MANAGEMENT. J. Piñeiro[‡], M. Maquivar[&], A. Barragan[‡], J. Velez[†], H. Bothe[†], and G. Schuenemann[‡] [‡]*Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA*
[&]*Department of Animal Sciences, Washington State University, Pullman, WA*
[†]*Aurora Organic Farms, Boulder, CO, USA*

The objective was to assess the effect of postpartum uterine diseases on milk yield (kg), milk components (SCC and percent fat and protein), and culling up to 300 days in milk (DIM). Cows (n = 3,227) from 2 dairies were screened for retained placenta (RP; > 24 h after parturition), metritis (within 20 d in milk [DIM]), and purulent vaginal discharge (PVD) at 26 ± 3 DIM. Weekly, a list of cows by DIM was obtained using on-farm computer records and screened for RP (presence of fetal membranes outside the vulva), metritis (fetid brown-red watery vaginal discharge and fever), and PVD (gloved hand technique). Parity (lactations 1, 2 and ≥3) of cows was considered for milk yield, milk components, and culling. The statistical analyses were performed using SAS. Cows with metritis, RP or PVD had an additive effect on milk yield, milk components, and culling. Regardless of parity, lactating cows diagnosed with uterine diseases (all combined) had significantly reduced milk yield (by 2–3.9 kg/cow/d) for at least one of the first 4 DHIA test-days ($P < 0.05$), but was not different at later tests. For the first 2 DHIA test-days, lactating cows diagnosed with uterine disease (all combined) had significantly higher SCC (232×10^3 cells/mL) and fat content (3.7%) compared with cows without uterine diseases (164×10^3 cells/mL and 3.5%, respectively; $P < 0.05$). Milk protein content (%) was not different between cows with or without uterine diseases. Cows with uterine diseases had higher ($P < 0.05$) culling within 60 DIM and significantly lower ($P < 0.05$) pregnancy hazard up to 300 DIM compared with cows without uterine diseases, regardless of parity. Uterine diseases decreased milk yield and changed milk components early in lactation; and these diseases were a substantial risk factor within 60 DIM for culling.

Keywords: Organic, dairy cattle, uterine disease, milk yield, culling

DISTRIBUTION AND DIVERSITY OF *SALMONELLA* IN SHIPMENTS OF HATCHLING POULTRY, UNITED STATES, 2013-2015. A. Sharma¹, M.M. Erdman², L. Muñoz-Vargas¹, R. O'Shaughnessy¹, G.G. Habing¹. (1) The Ohio State University, (2) National Veterinary Services Laboratories, APHIS, USDA

Direct contact is an important route of transmission for non-typhoidal *Salmonella* in the United States. Every year, multiple outbreaks of salmonellosis are linked to contact with live poultry. This study describes the distribution and diversity of serotypes, genotypes, and antimicrobial resistance phenotypes of *Salmonella* recovered from shipped boxes of mail-order hatchling poultry in 2015, and makes comparison to the population of *Salmonella* in prior comparable studies conducted in 2013 and 2014. In 2015, employees of 50 feed stores from a single national chain with spring sales of hatchling poultry submitted hatchling pads, a questionnaire, and shipment tracking information from hatchling boxes to the investigators. A total of 552 hatchling pads from 298 shipment boxes were received and cultured for *Salmonella* between February and May 2015. Isolates were sent to the National Veterinary Services Laboratory (Ames, IA) for serotyping, pulsed-field gel electrophoresis and antimicrobial resistance testing. The PFGE patterns of isolates from hatchling boxes were compared with isolates from human outbreaks of non-typhoidal *Salmonella*. In 2015, the sample level and the box level prevalence of *Salmonella* was 19.9% (110/552) and 27.2% (81/298), respectively. Of the recovered isolates, 18 different serovars and 36 different PFGE patterns were identified, including 5 serovars and 10 PFGE patterns that were indistinguishable from strains linked to concurrent human outbreaks of salmonellosis associated with contact with live poultry. Fourteen of 110 isolates (12.7%) isolates were resistant to cephalosporins. Relative to comparable studies in prior years, the prevalence of *Salmonella* Enteritidis and *Salmonella* Kentucky increased from 2013 to 2015. Additionally, the proportion of isolates resistant to >2 classes of antimicrobials increased in 2014 and 2015 compared to that in 2013. The results indicate a need to strengthen *Salmonella* control measures in hatcheries and create awareness of zoonotic transmission of the pathogen among backyard poultry owners.

Keywords: salmonella, zoonotic transmission, poultry, antimicrobial resistance, outbreak, chicks

DETECTION OF *HAMMONDIA HEYDORNI* OOCYSTS IN WILD AND DOMESTIC CANID FECES. D. Sinnott, K. Moreno Torres, B. Wolfe, R. Garabed, and A. E. Marsh. Dept. of Veterinary Preventive Medicine.

The coccidian parasite *Hammondia heydorni* is a close relative of and morphologically indistinguishable from *Neospora caninum*, and oocyst shedding of both parasites has been documented in several canid species. This study aimed to identify *H. heydorni* oocysts in the feces of wild canids and a domestic dog. Two hundred and eighty-five wild canid fecal samples were analyzed in addition to a domestic canine patient presenting to The Ohio State University Veterinary Medical Center. PCR with melting curve analysis was used to detect coccidian DNA. Coccidia-positive samples were further subjected to a *H. heydorni*-specific PCR assay targeting the ITS-1 region and a *N. caninum*-specific PCR assay targeting the Nc5 gene. Samples positive by the *H. heydorni*-specific assay were also analyzed with a PCR assay targeting the alpha tubulin gene to distinguish *H. heydorni* from *Hammondia triffittae*. *Hammondia heydorni* was detected in 3 wildlife samples (1.1%) as well as the dog sample. All samples were negative for *N. caninum*. The coccidia-specific, *H. heydorni*-specific, and *N. caninum*-specific assays were tested against several other coccidia species to assess their analytic specificity. Determining the presence of *H. heydorni* in wild canids will contribute to a greater understanding of the role these hosts play in the disease ecology of this parasite.

Keywords: *Hammondia*, fecal, coccidia, wild canids, dog, PCR

EVALUATION OF RISK FACTORS AND TRANSMISSION PATHWAYS OF SALMONELLA AND ESβL-PRODUCING ORGANISMS FOR DOGS ON OHIO LIVESTOCK FARMS. A. Smith, N. Moran, T. Mills, D. Mollenkopf, D. Mathys, T. Wittum, and J. Stull. Dept. of Veterinary Preventative Medicine.

Livestock, the environment, humans, dogs and their diets are all theorized to be involved in the transmission of *Salmonella* spp. and extended-spectrum-beta-lactamase (ESβL)-producing organisms on livestock farms. Dogs may play a unique role due to their many on-farm roles and high human and livestock contacts. This study aimed to determine the prevalence of *Salmonella* and ESβL-producing organisms (*bla*_{CTX-M} and *bla*_{CMY-2}) in dogs on Ohio livestock farms and identify likely domains (farm environment/livestock, dog diet, humans, other dogs) involved in the transmission of these organisms to dogs. Surveys gathering individual dog-level exposures and overall farm husbandry were collected from 71 livestock farmers, along with fecal samples from 100 dogs on these farms. Dog samples were tested for *Salmonella* spp. and ESβL-producing organisms. Survey data were categorized into domains to identify significant predictors (within and between domains) for dog carriage of *Salmonella* and ESβLs. Seven percent of dogs were shedding *Salmonella* spp., and 4% and 39% *Escherichia coli* carrying *bla*_{CTX-M} and *bla*_{CMY-2}, respectively. Multivariable logistic regression models for each pathogen identified significant domain-associated predictors of dog carriage: *Salmonella* (farm domain): farms for personal use (OR=0.1), dog access to livestock bedding (OR=13); *bla*_{CTX-M} (farm domain): meals fed in farm building (OR=23), presence of swine (OR=18); *bla*_{CMY-2} (farm domain): working dogs (OR=22), goat exposure (OR=4), presence of goats (OR=9), (dog diet domain): fed raw diet (OR=3), (human domain): enrolled through 4H recruitment (OR=4), dogs allowed to sleep in all locations in house (OR=9), dogs go on car rides (OR=21), (other dogs domain): multiple dogs on the farm (OR>33). This study demonstrated the likely on-farm transmission of these pathogens to dogs from multiple domains, highlighting the need for dynamic, multi-domain approaches to pathogen canine prevention and control on livestock farms.

Keywords: dogs, livestock, *Salmonella*, *E. coli*, extended-spectrum-beta-lactamase (ESβL)-producing organisms, antimicrobial resistance, infection control, biosecurity, zoonoses

ASSESSMENT OF THE CANINE RABIES PROGRAM IN ETHIOPIA: A PROJECT OF RIGHT PARTNERSHIP'S PILOT PROGRAM IN NORTH GONDAR Waibel, S., O'Quin, J., and Gebreyes, W. The Department of Veterinary Preventative Medicine

Rabies is a deadly viral disease that is almost always fatal unless timely post-exposure prophylaxis is initiated. This disease is entirely preventable yet remains a significant public health risk in developing countries. Ethiopia has the highest reported incidence of rabies in Africa (1.6 deaths /100,000 population) causing at least 1,456 human fatalities/year. Domestic dogs are the natural reservoir and are responsible for approximately 95% of human rabies cases. To address this concern, The Rabies and Infections on Global Health in the Tropics (RIGHT) partnership was established in 2012. The goal is to operationalize One Health and devise a reproducible yet versatile approach to building rabies prevention and control programs. To accomplish this goal a systematic review of the current rabies surveillance and monitoring program in Ethiopia was conducted. The report includes an in-depth analysis of current protocols and practices at the national level at the Ethiopian Public Health Institute (EPHI), in Addis Ababa, as well as an implementation review at the local level in North Gondar. Data collection involved interviewing officials and reviewing records from the following organizations/agencies: EPHI (1), local Gondar veterinary clinics (4), Gondar healthcare centers (3), a hospital (1), the Gondar Public Health Department (1), and the Amhara Zonal Agricultural Office (1). Comparison of the national protocols to the current practices at the local level identified infrastructure gaps. Similarly, comparison of the current program to the proposed RIGHT plan further identified specific needs to be addressed. Key results include: 1) None of the clinics (0/4) had appropriate safety equipment for restraining potentially rabid dogs or staff who were vaccinated against rabies, 2) major preventive vaccine shortage for both dogs and humans 3) funding and technology deficiencies promote fragmented reporting 4) attempted treatment by traditional healers may underestimate the true incidence of human rabies cases.

Keywords: canine rabies, Ethiopia

**IMMUNOLOGY
AND
INFECTIOUS DISEASES**

IMID-1

SAMHD1-MEDIATED HIV-1 RESTRICTION IN CELLS DOES NOT INVOLVE RIBONUCLEASE ACTIVITY. JM Antonucci^{1, 2}, C St. Gelais¹, S de Silva¹, JS Yount³, C Tang⁴, X Ji⁴, C Shepard⁵, Y Xiong⁴, B Kim⁵, L Wu¹⁻³

¹ Center for Retrovirus Research, Department of Veterinary Biosciences; ² Department of Microbiology; ³ Department of Microbial Infection and Immunity; The Ohio State University, 1900 Coffey Road, Columbus, OH; ⁴ Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT; ⁵ Department of Pediatrics, Center for Drug Discovery, Emory University School of Medicine, 1760 Haygood Drive, Atlanta, GA.

SAMHD1 restricts HIV-1 replication in non-dividing cells by degrading dNTPs to a level that limits efficient HIV-1 reverse transcription (RT). It's been reported that SAMHD1 acts as an RNase and restricts HIV-1 replication in non-dividing cells through degradation of viral genomic RNA (gRNA), which challenged the established mechanism of HIV-1 restriction. To clarify these conflicting results, we independently generated stable U937 cell lines expressing SAMHD1 wild-type (WT) and mutants purported to specifically retain dNTPase (Q548A) or RNase (D137N) activities. Our results show WT SAMHD1 and the two mutants equally restricted HIV-1 infection and decreased dNTP levels in differentiated U937 cells. To determine whether SAMHD1 degrades HIV-1 gRNA, we measured HIV-1 gRNA levels in HIV-1-infected cells. We found similar levels of HIV-1 gRNA among the three SAMHD1-expressing cell lines compared to the vector control cells, indicating SAMHD1 does not degrade HIV-1 gRNA. Furthermore, we measured the levels of HIV-1 late RT products in infected cells and observed a significant decrease relative to vector control cells, suggesting SAMHD1 restricts HIV-1 infection at the level of RT. This correlates with the reduced dNTP pools measured in SAMHD1-expressing cell lines. Overexpression of SAMHD1 in virus producer cells didn't affect HIV-1 Gag expression, viral release or infectivity, suggesting that SAMHD1 does not degrade HIV-1 mRNA. To clarify whether SAMHD1 has a nuclease activity, we measured the ability of stringently purified full-length recombinant SAMHD1 to degrade ssDNA and ssRNA *in vitro*. All SAMHD1 preparations maintained a robust dNTPase activity; however, only background nuclease activity was observed in some preparations, indicating that the inconsistency of RNase activity is likely due to contamination. Overall, our data indicate that Q548A and D137N mutants of SAMHD1 do not distinguish the dNTPase and RNase function, and that dNTP hydrolysis is the most likely mechanism of SAMHD1-mediated HIV-1 restriction in non-dividing cells.

Keywords: SAMHD1, HIV-1, Restriction Factor, RNA

REGULATION OF IMMUNOGLOBULIN CLASS SWITCH BY PHARMACOLOGICAL INHIBITORS OF INFLAMMATION AND NEUTROPHIL FUNCTIONS. Z.Attia^{1,2}, H.E.Steiner¹, E.Kim¹, T.L.Martin¹, A.Zaghawa², E.Cormet-Boyaka¹, P.N.Boyaka¹. ¹Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, Columbus, OH; ²Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt .

Different immunoglobulin isotopes and subclasses play distinct roles in host protection against bacterial, viral and parasitic infections. Each immunoglobulin isotope and immunoglobulin subclass is produced after IgM-bearing B cells receive stimuli that allow immunoglobulin class and transcription of immunoglobulin γ , ϵ or α chain genes corresponding to IgG, IgE and IgA. It is now well established that the anti-inflammatory cytokine IL-10 and TGF- β promote immunoglobulin class switch and production of IgA, and our group has recently shown that depletion of neutrophils facilitates generation of IgA responses by experimental sublingual vaccines. Since vaccines can be given to animal or human undergoing treatment with pharmacological inhibitors of inflammation (i.e., aspirin) or neutrophil functions (i.e., sivelestat and sulfasalazine), we tested the effect of such inhibitors on IgG and IgA production *in vitro*. Our results show that addition of aspirin, sivelestat or sulfasalazine to culture of LPS-stimulated murine spleen cells down regulates expression of B220 by B cells while increasing the expression of the plasma cell marker Syndecan1. Interestingly, these pharmacological inhibitors has similar effect of plasma cell differentiation than cholera toxin, one of the most describe adjuvant for inducing IgA responses in experimental animals. Flow cytometry analysis of surface immunoglobulin on B cells and quantification of immunoglobulin secreted in culture supernatants further demonstrated that aspirin, sivelestat and sulfasalazine increase production of both IgG and IgA. Taken together, our these findings suggest that addition of inhibitors of inflammation or neutrophil function to vaccine may represent a potential strategy for fine tuning immune response to vaccines and promoting mucosal IgA responses.

Keywords: Immunoglobulin class switch, inflammation, neutrophils, IgA

RANDOM MUTAGENESIS OF *EHRLICHIA* SP. HF STRAIN FOR IDENTIFICATION OF VIRULENCE GENES. H. Bekebrede, M. Lin, Y. Rikihisa. Dept. of Veterinary Biosciences

Ehrlichia spp. (*E. canis*, *E. ruminantium*, *E. ewingii*, and *E. chaffeensis*) are tick-borne obligatory intracellular bacteria that infect variety of mammals including dogs, ruminants, deer, and human, causing severe and sometimes fatal systemic disease. Research to identify virulence factors of *Ehrlichia* spp. is hampered by the lack of small laboratory animal models and is inaccessible to stable genetic manipulations. The Rikihisa laboratory isolated a novel *Ehrlichia* species named “HF strain” from ticks in Japan. The HF strain is most closely related to *E. chaffeensis* human isolates, and notably kills laboratory mice in 10 days. The Rikihisa laboratory also recently completed whole genome sequencing of the HF strain. My research seeks to analyze gene function of the HF strain by using Himar transposon mutagenesis. A random mutant HF strain library will be created in canine macrophage cell line, DH82 cells. Mutant HF strains will be cloned, and genomic loci of transposon insertion will be identified by inverse PCR. Isolated mutants that can disrupt the promoter region or open reading frame will be confirmed by RT-PCR for the lack of mRNA. Ten distinct mutants will be selected to determine effects of the mutant HF strain on mice pathogenesis. I have so far isolated a single stable mutant clone that expresses mCherry fluorescence. The transposon insertion site was determined to be in the intergenic region between EHF_0098 and EHF_0097. Currently, we are seeking to obtain more mutants suitable for *in vitro* and *in vivo* pathogenesis analysis with optimal plasmid preparation and transformation methods. These studies will be expected to elucidate virulence factors of the HF strain. Because *Ehrlichia* spp. share homologous genes, the proposed study will help understanding virulence factors of other *Ehrlichia* spp. as well.

Keywords: Ehrlichia, HF strain, obligate intracellular bacteria, virulence, mutagenesis

POLY-LACTIC-CO-GLYCOLIC ACID (PLGA) NANOPARTICLE DELIVERY OF SWINE INFLUENZA VIRUS VACCINE PROVIDES HETEROLOGOUS PROTECTION THROUGH CELL MEDIATED IMMUNITY IN PIGS. S. Dhakal, J. Hiremath¹, K. Bondra¹,

Y. SL¹, B. Shyu¹, K. Oyuang¹, B. Binjawadagi¹, K.I. Kang¹, J. Goodman², B. Narasimhan², C.W. Lee¹, R.J. Gourapura¹; ¹Food Animal Health Research Program, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA, ²Department of Chemical and Biological Engineering, Iowa State University, Ames, IA, USA.

Swine influenza is one of the major economic burdens to swine farmers in US. Current vaccines have failed to provide heterologous (cross) protection, warranting the need of innovative vaccine delivery platform. PLGA is a biodegradable polymer, Food and Drug Administration approved, and widely used in drug and vaccine delivery system. In this study, PLGA nanoparticle containing killed swine influenza virus (SwIV) H1N2 (KAg) vaccine (PLGA-KAg) was developed; and evaluated *in vitro* and *in vivo* in a typical vaccination and heterologous SwIV H1N1 challenge trial in pigs. Our results showed that PLGA-KAg induced maturation of antigen presenting cells *in vitro*. In pigs intranasally vaccinated with PLGA-KAg, at 35 days post-vaccination, increased frequencies of cytotoxic T cells (CTLs), memory T helper cells and gamma-delta T cells, and enhanced antigen specific proliferation of lymphocytes were observed. After heterologous virus challenge in PLGA-KAg vaccinated pigs: (i) absence of clinical flu symptoms like fever, anorexia and lethargy; (ii) significantly reduced virus induced lung pathology and antigenic mass; (iii) significantly higher CTLs and total interferon gamma producing cells; and (iv) virus clearance in the respiratory tract of most of the pigs compared to KAg vaccinated animals was observed. But PLGA-KAg vaccine failed to boost the antibody response both in pre- and post-challenged pigs. In summary, our study showed the particulate delivery of killed SwIV vaccine induced protective immune response was mediated through cellular (CTLs) but not humoral (antibody) immune response in pigs. Upon a few important improvements to this vaccine delivery platform, it can serve as a potent candidate vaccine to use in swine herds to mitigate flu outbreaks.

Keywords: Swine influenza, Poly-lactic-co-glycolic acid, Nanoparticle, Pig, Vaccine

THE EFFECT OF HYPOTHERMIA ON INFLUX OF MONONUCLEAR CELLS IN THE DIGITAL LAMELLAE OF HORSES WITH OLIGOFRUCTOSE-INDUCED LAMINITIS. J.D. Godman¹, T.A. Burns¹, C.S. Kelly¹, M. Watts¹, B.S. Leise², E.L. Schroeder¹, A.W. van Eps³, J.K. Belknap¹ 1. The Ohio State University, Columbus OH, 2. Louisiana State University, Baton Rouge, LA, 3. The University of Queensland, Brisbane, Australia

Sepsis-related laminitis (SRL) is a common complication in the septic/endotoxemic critically-ill equine patient. Similar to organ injury in human sepsis, lamellar injury in SRL has been associated with inflammatory events, including the influx of leukocytes into the lamellar tissue and markedly increased expression of a wide array of inflammatory mediators at the onset of Obel grade 1 (OG1) laminitis. The only treatment reported to protect the lamellae in SRL, local hypothermia, has been demonstrated to effectively inhibit lamellar expression of multiple inflammatory mediators. However, the effect of hypothermia on leukocyte influx into affected tissue has not been assessed. We hypothesized that hypothermia inhibits leukocyte emigration into the digital lamellae in SRL.

Immunohistochemical staining using leukocyte markers MAC387 (neutrophils, activated monocytes) and CD163 (monocyte/macrophage-specific) was performed on archived lamellar tissue samples from a carbohydrate overload model. One forelimb was maintained at ambient temperature (AMB) and one forelimb was immersed in ice water (ICE) immediately following oligofructose administration (10g/kg, n=14 horses). Lamellae were harvested at 24 hours post-oligofructose administration (DEV, n=7) or at the onset of OG1 laminitis (OG1, n=7). Leukocytes were counted by a single blinded investigator on images [n=10 (20x fields/digit for MAC387; 40x fields/digit for CD163)] obtained using Aperio software. Data were assessed for normality and analyzed with a paired t-test and one-way ANOVA with significance set at p<0.05. MAC387(+) cells were present in low numbers in the lamellar tissue and were decreased in the hypothermic limbs (vs. AMB limbs, p<0.05) in the OG1 group; no change in CD163(+) cell numbers was noted.

This study demonstrated that hypothermia of the distal limbs instituted early in the disease process in the horse at risk of SRL significantly attenuates the increase of MAC387(+) leukocytes in the digital lamellae, but has minimal effect on increases in lamellar concentrations of CD163(+) mononuclear cells.

Keywords: Laminitis, sepsis, hypothermia, CD163, MAC387, oligofructose

CX3CR1 IN COTTON RATS IS THE RECEPTOR FOR RESPIRATORY SYNCYTIAL VIRUS AS IT IS IN HUMANS. G Green¹, S. Johnson², A. Oomens⁴, M. Teng³, M. Peeples², S. Niewiesk¹

¹Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio; ²Center for Vaccines and Immunity, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio; ³Division of Allergy and Immunology, Department of Internal Medicine, Morsani College of Medicine, University of South Florida, Tampa, Florida; ⁴Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, Oklahoma.

Respiratory syncytial virus (RSV) is a leading cause of death among infants worldwide. Despite this, there are no effective vaccines or antivirals available. A recent publication by SM Johnson et. al. [(2015) RSV Uses CX3CR1 as a Receptor on Primary Human Airway Epithelial Cultures. PLoS Pathog 11(12):e1005318], has demonstrated that RSV uses CX3CR1 rather than heparan sulfate as a receptor on primary human airway epithelial cell cultures, a more physiologically relevant model than immortalized cell lines, which express heparan sulfate as a receptor. RSV replicates in the upper and lower respiratory tract of cotton rats in a manner similar to humans. Sequencing and cloning of cotton rat CX3CR1 revealed 82% shared amino acid sequence identity with humans. RSV binds to CX3CR1 via its attachment glycoprotein or G protein. In order to understand the interaction of RSV with its receptor molecule in vivo, we used G protein mutated in the CX3CR1 binding site and measured viral replication in the lungs of cotton rats following intranasal inoculation. All virus mutants grew well in cell culture on immortalized cell lines, but in contrast to wild-type virus, the G protein mutants were not detectable at 4 days post-infection in the lung. Wild-type RSV was recovered at a titer of $10^{4.3}$ TCID₅₀/gram lung tissue. In a similar experiment, RSV was incubated with an antibody directed against the CX3CR1 binding site of G protein to block G protein binding to CX3CR1. Subsequent intranasal inoculation into cotton rats resulted in undetectable levels of RSV in the lungs 4 days post-infection. If RSV was incubated with heparan sulfate before intranasal inoculation into cotton rats, viral growth was not affected. So far, these results indicate that CX3CR1 functions as the receptor for RSV in cotton rats. Experiments are in progress to formally prove this role of CX3CR1.

Keywords: Respiratory Syncytial Virus, Viral entry, Receptor, Cotton rat

EVALUATION OF THE VIRULENCE OF A PORCINE EPIDEMIC DIARRHEA VIRUS WITH A 197 AMINO ACID-DELETION IN THE SPIKE PROTEIN

Y. Hou, C.M Lin, X. Gao, Z. Lu, X. Liu, Y. Qin, L. J. Saif and Q. Wang

Food Animal Health Research Program, Ohio Agricultural Research and Development Center, College of Food, Agricultural and Environmental Sciences, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, Ohio, USA

Porcine epidemic diarrhea virus (PEDV) belongs to the *Alphacoronavirus* genus within the *Coronaviridae* family. It is a highly contagious and lethal enteric pathogen in piglets. A mutant PEDV strain PC177 shares a similar backbone to the highly virulent original US PEDV strain PC22A, but has a 197 amino acid (aa)-deletion in the N-terminal domain (NTD) of the S1 region of the spike (S) protein. For the alphacoronavirus transmissible gastroenteritis virus (TGEV), a 224 aa-deletion in the NTD of S1 reduced its virulence and changed its tissue tropism from intestinal to respiratory [subsequently designated porcine respiratory coronavirus (PRCV)]. We hypothesized that the 197 aa deletion of PC177 may alter its virulence and tissue tropism. To test this hypothesis, 4-day-old cesarean-derived colostrum-deprived (CDCD) piglets were inoculated orally with PC177 (n=6), PC22A (n=5), or mock (n=4). Within 7 days post-inoculation (DPI), no mock pigs had diarrhea, but 100% PC22A- and 50% PC177-inoculated piglets had diarrhea. However, PC177-inoculated piglets showed milder diarrhea and lower fecal PEDV RNA shedding titers compared with PC22A-inoculated piglets. Mortality rates were 0% and 100% in PC177- and PC22A-inoculated piglets, respectively. Immunohistochemistry (IHC) staining of PEDV N protein at the acute phase of infection showed that both PC177- and PC22A-antigens were detected in the small intestinal epithelial cells, but not in the bronchial epithelial cells and lungs. In addition, milder villous atrophy and lower antigen scores were observed in PC177-inoculated piglets. In a recent report, no mortality occurred in 7-day-old field suckling piglets (n=120) infected with a Japanese PEDV strain (Tottori2/2014) with a 194 aa-deletion (Δ 23-216 aa) in the S1 NTD, similar to that of PC177 (Δ 34-230 aa). Both findings suggest that the large deletion in the NTD of S1 may be responsible for the reduced virulence.

Keywords: porcine epidemic diarrhea virus, spike, pathogenesis

CATHEPSIN K INHIBITION RENDERS EQUINE BONE MARROW NUCLEATED PROGENITOR CELLS HYPO-RESPONSIVE TO LPS AND UNMETHYLATED CPG STIMULATION *IN VITRO*. H. Hussein¹, P. Boyaka², J. Dulin¹, A. Bertone^{1, 2}. ¹.Dept. of Veterinary Clinical Sciences. ². Dept. of Veterinary Biosciences. College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.

Cathepsin K (CatK) is an important enzyme regulating bone degradation and immune response. Cathepsin K inhibition has been proposed as a therapeutic for equine osteo-inflammatory conditions. Bone marrow is the body's resource for progenitor cells of osteoblastic and osteoclastic cell lines responsible for bone formation and turnover, and for the lymphoid cell lines as well. Our study aimed to investigate the effect of CatK inhibition on Toll like receptor (TLR) 4 and TLR9 signaling pathways in equine whole bone marrow nucleated cells (BMNCs). This cellular fraction was chosen to include both the lymphoid and non-lymphoid cells (myeloid progenitors, mesenchymal stem and other progenitor cells) since equine immune (myeloid and lymphoid) and non-immune cells, such as chondrocytes and synovial fibroblast-like cells showed significant inflammatory response when stimulated with Lipopolysaccharides (LPS) *in vitro*. Equine BMNCs were isolated and exposed to VEL-0230, a highly selective CatK inhibitor, at a concentration of 0, 1, and 10 μ M in cell culture media with and without LPS (1 μ g/ml) and unmethylated CpG (5 μ g/ml). Subsequent analyses of cell viability, cytokine secretion by stimulated BMNCs; specifically IL-1 β , IL-6, and TNF- α , and BMNCs surface markers' expression and Major histocompatibility (MHC) II molecule were performed. Cathepsin K inhibition promoted BMNCs viability and reduced cell apoptosis. Moreover, CatK inhibition significantly decreased cytokine secretion and MHC II molecules expression of either naïve or stimulated BMNCs. In conclusion, CatK inhibition in horses did affect BMNCs other than mature osteoclasts rendering them hypo-responsive to both TLR4- and TLR9-induced inflammation, predicting a proteolytic activity for CatK within the MyD88 pathway and/or the following proteolytic events required for the cytokines secretion.

Keywords: immune, cytokine, inflammation, Toll like receptor, Cathepsin K, VEL-0230.

ANTIMICROBIAL USE AND RESISTANCE IN ZOONOTIC BACTERIA RECOVERED FROM NONHUMAN PRIMATES. J. Kim, The Ohio State University, College of Veterinary Medicine, Department of Veterinary Preventive Medicine, College of Public Health, Columbus, Ohio, United States of America; D. J. Coble, The Ohio State University, College of Veterinary Medicine, Department of Veterinary Preventive Medicine, University of Laboratory Animal Resources, Columbus, Ohio, United States of America; G. W. Salyards, University of California, Davis, California National Primate Research Center, Davis, California, United States of America; W. Rinaldi, Alpha Genesis Incorporated, Yemassee, South Carolina, United States of America; G. Plauche, University of California, Davis, California National Primate Research Center, Davis, California, United States of America; G. H. Habing, The Ohio State University, College of Veterinary Medicine, Department of Veterinary Preventive Medicine, Columbus, Ohio, United States of America

Antimicrobial resistance (AMR) has become a central topic as it is a growing threat in human and animal health. Major surveillance systems, such as the National Antimicrobial Resistance Monitoring System (NARMS), are now established to monitor AMR. However, there appears to be a lack of comprehensive literature on AMR among nonhuman primates (NHP) used in biomedical research. *Shigella flexneri*, *Yersinia enterocolitica*, *Y. pseudotuberculosis*, and *Campylobacter jejuni* are zoonotic enteric bacteria common among NHPs, and AMR strains may lead to serious mortality and morbidity in both NHP patients in staff. This study aims to provide data on current antimicrobial use strategies and the prevalence of AMR in zoonotic bacteria recovered from NHPs within biomedical research institutions. Fifteen veterinarians, seven biomedical institutions, and four diagnostic laboratories participated, providing data on antimicrobial practices and susceptibility test results across three years (1/2012 – 4/2015). Participating veterinarians also identified a threshold prevalence of AMR (TP-AMR), where if exceeded by the true prevalence of AMR, would cause the veterinarians to change their antimicrobial use strategies. We hypothesized that the prevalence of AMR among the above bacteria will exceed participating veterinarians' TP-AMRs. Participating veterinarians primarily treated cases caused by *S. flexneri*, *Y. enterocolitica*, and *Y. pseudotuberculosis* with enrofloxacin, but treated *C. jejuni* cases with azithromycin and tylosin. High proportions of AMR were observed to other antimicrobials, but all isolates were susceptible to their associated primary antimicrobials. Notably, resistance patterns were not shared between this study's NHP isolates and human isolates presented by NARMS. The presented study demonstrates that zoonotic bacteria recovered from NHP diagnostic samples are broadly susceptible to the antimicrobials used to treat the clinical infections. These results can help veterinarians ensure effective antimicrobial therapy and consequently, protect staff by minimizing occupational risk.

Keywords: *Shigella flexneri*; *Yersinia enterocolitica*; *Yersinia pseudotuberculosis*; *Campylobacter jejuni*; antimicrobial resistance (AMR); nonhuman primates (NHP); threshold prevalence of AMR (TP-AMR); National Antimicrobial Resistance Monitoring System (NARMS)

EPITHELIAL CELL IKK β REGULATES EOSINOPHIL LEVELS IN THE INTESTINE AND SEVERITY OF ALLERGIC RESPONSES TO INGESTED ALLERGENS E. Kim, M. M. Lember, T. L. Martin, J. C. Rowe, H. E. Steiner, E. Cormet-Boyaka, P. N. Boyaka. Depts. of Veterinary Biosciences

Allergic sensitization to food allergens has subsequent potential to developing allergic responses in the gastrointestinal tract, and also to skin or lung. Our previous study showed that lack of IKK β in intestinal epithelial cells regulates favors IgA responses to ingested allergen, which in turn limits the severity of allergic responses in the airway. In this study we investigated whether intestinal epithelial IKK β also regulated allergic responses to oral antigens. Wild-type C57BL/6 and IKK $\beta^{\Delta IEC}$ mice, which lack IKK β in intestinal epithelial cells, were orally sensitized to a food antigen in the presence of cholera toxin. Allergen-specific serum IgE responses and fecal IgA responses were similar between the groups. However, after oral allergen-challenge, IKK $\beta^{\Delta IEC}$ mice only developed minimal clinical and histological signs of allergy, including drop in body temperature and mucus in small intestinal villi and crypts. Interestingly, IKK $\beta^{\Delta IEC}$ mice expressed lower levels of CCL11 (eotaxin) and eosinophils than control wild-type mice and their levels were only weakly increased after oral allergen sensitization and challenge. In summary, this study reveals a new role of intestinal epithelial cells in the regulation of allergy in the GI tract through a NF- κ B – CCL11 axis.

Keywords: Allergy, Intestinal epithelium, IKK β , Eosinophil, CCL11

DEVELOPING A CRYOPRESERVATION METHOD THAT PRESERVES FUNCTION OF CANINE AND FELINE PERIPHERAL BLOOD MONONUCLEAR CELLS. Y. Lin, R.

Vicetti Miguel, N. Quispe Calla, K. Henschel, and T. Cherpes. Depts. of Microbial Infection and Immunity and Obstetrics and Gynecology

Adequate cryopreservation methods for canine and feline peripheral blood mononuclear cells (PBMC) do not exist. Herein, we compared viability and function of canine and feline PBMC using the current gold standard for human PBMC cryopreservation (i.e., 90% fetal bovine serum (FBS) and 10% dimethyl sulfoxide (DMSO)) vs. a serum-free medium (i.e., RPMI supplemented with 12.5% bovine serum albumin (BSA) fraction V and 10% DMSO). Though FBS-based media consistently preserved the number and viability of cryopreserved canine PBMC (i.e., 90% cell recovery after thaw), recovered cells were less responsive than fresh cells to polyclonal immune activators. Conversely, serum-free media allowed 80% recovery of cryopreserved canine PBMC, but better conserved responsiveness to immune activation. To directly define capacity of the serum-free medium to preserve immune function of cryopreserved vs. fresh PBMC, we isolated fresh feline PBMC and stimulated them with polyclonal immune activators, and also cryopreserved an aliquot of these same PBMC in serum-free media. One week later, frozen PBMC were recovered and stimulated identically as the fresh PBMC. Comparing these responses, we newly demonstrate that serum-free medium preserves immune function of feline PBMC. Our optimization of methodology that preserves immune function of canine and feline PBMC is likely to significantly impact immunological studies in comparative oncology, infectious disease pathogenesis in preclinical models of human disease, and companion animal vaccine development.

Keywords: PBMC, peripheral blood mononuclear cells, cryopreservation, serum-free, immune function, canine, feline

EXPERIMENTAL MODELING OF THE NONSPECIFIC PROTECTIVE EFFECTS WITH MEASLES VIRUS VACCINATION. S. C. Linn, D. Huey, and S. Niewiesk. Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University

The administration of a vaccine can have non-specific effects that are protective against unrelated pathogens in an infant patient and can, therefore, be protective against viruses for which currently no vaccines exist. Respiratory syncytial virus (RSV) is one of the most common causes of lower respiratory tract infections in infants with 3.4 million cases leading to hospitalization in children under 5 years of age. A recent study in Denmark, however, observed that children whose most recent vaccine was the live measles-mumps-rubella vaccine had a lower rate of RSV hospitalization compared to children who had inactivated DTaP-IPV-Hib3 as their most recent vaccine. The aim of this study is to provide the experimental basis for the nonspecific protective effects of measles virus immunization in the presence or absence of maternal antibodies against infection with RSV. We established three groups of animals: cotton rats immunized with measles virus in the presence or absence of measles virus specific maternal antibodies and unvaccinated controls. From these groups, we measured viral titers of RSV in lung and nasal turbinate homogenates and measured the antibody response against measles virus and RSV. We have analyzed RSV viral titers from 1, 3, and 5 weeks post vaccination with measles along with 5 weeks post-measles vaccination in the presence of maternal antibodies. For the three timepoints observed, there was no correlation between measles virus immunization and RSV infection in cotton rats.

Keywords: cotton rat, Respiratory Syncytial Virus, measles virus

3'3'-CGAMP INDUCES A BALANCED TH1 AND TH2 CYTOKINE PROFILE FOLLOWING SUBLINGUAL IMMUNIZATION. T. Martin, E. Kim, J. Jee, H.E. Steiner, and P.N. Boyaka. Dept. of Veterinary Biosciences

Mucosal immunization confers systemic immunity as well as immunity in mucosal compartments. This mucosal protection occurs through induction of secretory IgA antibodies (SIgA.) Intranasal immunization is a well-established route of mucosal immunization in both humans and animals, however adverse effects have been associated with this route. Facial nerve paralysis is reported in humans immunized with vaccines containing the adjuvants cholera toxin (CT) or heat-labile toxin from *Escherichia coli* (LT.) Mucosal immunization via the sublingual route could obviate this risk while effectively inducing SIgA, but currently approved adjuvants such as alum do not effectively induce SIgA following sublingual immunization. 3'3'-cyclic guanosine monophosphate-adenosine monophosphate (3'3'-cGAMP) is a cyclic dinucleotide of bacterial origin that elicits innate immune responses by binding to stimulator of interferon gamma genes (STING) on the endoplasmic reticulum. To examine the effectiveness of 3'3'-cGAMP as an adjuvant for sublingual immunization, C57BL/6J mice were immunized with recombinant *Bacillus anthracis* protective antigen (PA) in the presence of 3'3'-cGAMP. Sublingual immunization with 3'3'-cGAMP elicited a balanced Th1 and Th2 immune response, as evidenced by the ratio of serum IgG2a:IgG1. This response was further evidenced by the cytokine profile elicited by 3'3'-cGAMP immunization, which included elevations in both pro- and anti-inflammatory cytokines, but did not lead to elevations in production of Th2 or Th17 cytokines associated with allergies.. Combined with previous data showing that immunization with 3'3'-cGAMP encourages SIgA production in the respiratory tract, this data indicates that 3'3'-cGAMP could be both a safe and effective adjuvant for sublingual immunization against respiratory pathogens such as *Bacillus anthracis*.

Keywords: sublingual, mucosal, immunization, adjuvant, Immunoglobulin A, IgA, cGAMP

MATERNAL ANTIBODY TRANSFER IN THE COTTON RAT PLACENTA

M. E. Martinez¹, K. M. D. La Perle^{1,2}, S. Niewiesk¹

¹Department of Veterinary Biosciences and the ²Comparative Pathology and Mouse Phenotyping Shared Resource, The Ohio State University, Columbus, Ohio

Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis and viral pneumonia in infants and young children worldwide, and a significant cause of respiratory disease in the elderly. There is no vaccine or antiviral therapy to prevent or treat RSV infection, but virus neutralizing monoclonal antibodies can be given prophylactically emphasizing the protective potential of antibodies. One concept of RSV vaccinology is the immunization of mothers to induce high antibody titers, which will lead to the transfer of high levels of maternal antibodies. Currently, there are clinical trials investigating the benefit of maternal immunization for RSV to induce protective passive immunity in infants. Cotton rats are the best small animal model for RSV infection and are used to test maternal immunization. In cotton rats, maternal IgG antibodies is transferred via the placenta in utero and postnatally via intestinal absorption from colostrum. To further develop the cotton rat model, we characterized the cotton rat placenta and Fc receptor localization. Placentas from cotton rats at mid-gestation (~ day 14) and late gestation (~ day 27), as well as neonatal (<1 week) gastrointestinal tracts were collected for light microscopy, immunohistochemistry and electron microscopy. The cotton rat placenta is hemotrichorial, and has 5 distinct layers: decidua, junctional zone, labyrinth, chorionic plate, and yolk sac. Consistent with the transfer of maternal antibodies, the majority of the Fc receptors were present in the yolk sac endoderm, as well as the chorionic plate fetal capillary endothelium, and 10% of the fetal capillary endothelium within the labyrinth. Fc receptors were also present in the duodenal and jejunal enterocytes, similar to humans, mice, and rats. Therefore, the cotton rat is an animal model that can be used to investigate maternal antibody transfer after maternal immunization to prevent RSV infection in infants.

Keywords: Cotton rat, Placenta, Fc receptor, Passive transfer

EFFECT OF NF- κ B PATHWAY IN INTESTINAL EPITHELIAL CELLS DURING INGESTION OF LOW DOSES OF CADMIUM. J. Rowe, E. Kim, H. Steiner, E. Cormet-Boyaka, and P. Boyaka. Dept. Veterinary Biosciences

Chronic gastrointestinal inflammation is a great concern to human medicine, veterinary medicine, and food animal production. Minor disruptions to the homeostatic equilibrium of the intestinal tract can lead to Inflammatory Bowel Disease (IBD), Crohn's disease, and ulcerative colitis, which affect approximately 1.4 million people in the United States. Further, prolonged bouts of inflammation could similarly cause IBD in dogs and are contributing factors to colic in horses and depressed production of food animals. Low levels of cadmium are commonly found in water runoff and accumulation can occur in plants, seafood, and soft tissues of mammals. This heavy metal is now listed 7th in the priority list of hazardous substances and is believed to promote inflammation. This study explored the role of intestinal epithelial cells (IECs), and more specifically the canonical NF- κ B pathway of these cells, in host mucosal responses to repeated ingestion of cadmium. Control wild-type C57BL/6 and IKK $\beta^{\Delta IEC}$ mice, which lack IKK β in IECs, were maintained in conventional SPF housing (n=5 per group) and provided cadmium as CdCl₂ (10 uM or 2 ppm) in drinking water for 14 days. Analysis of total sIgA in fecal samples collected on days 0, 7, and 14 showed that cadmium treatment reduces sIgA levels in both groups of mice. We also found that repeated ingestion of cadmium differentially affected the frequency of lymphocyte subsets in mesenteric lymph nodes (MLNs) of IKK $\beta^{\Delta IEC}$ and control wild-type mice and increased percentage of B cells while reducing the percentage of T cells in IKK $\beta^{\Delta IEC}$ mice. Furthermore, cadmium treatment enhanced gut TGF β and TNF α mRNA responses to the bacterial product cholera toxin in IKK $\beta^{\Delta IEC}$ mice. Taken together, our data suggest that the canonical NF- κ B in intestinal epithelial cells plays a key role in host response to environmental pollutants and subsequent inflammatory status in the gastrointestinal tract.

Keywords: NF- κ B, Intestinal Epithelium, Cadmium

EXAGGERATED PRO-INFLAMMATORY INNATE IMMUNE RESPONSE OF CYSTIC FIBROSIS AIRWAY EPITHELIAL CELLS TO H1N1 INFLUENZA A INFECTION. S. YOUNG¹, P. Woods¹, M. Peeples², and I. Davis¹. ¹The Ohio State University College of Veterinary Medicine; ²Nationwide Children's Hospital Medical Center

Cystic Fibrosis (CF) is an autosomal recessive genetic disorder resulting from mutations in the cystic fibrosis transmembrane conductance regulator gene (*cftr*). CF is characterized by a progressive decline in lung function, often as a result of repeated pulmonary exacerbations. Relative to normal subjects, respiratory epithelial cells from CF patients release higher amounts of pro-inflammatory cytokines and chemokines following infection with bacterial pathogens such as *Pseudomonas aeruginosa*, which results in significant damage to the respiratory tract. This damage leaves the lung susceptible to further bacterial colonization, resulting in a vicious cycle of bacterial colonization, inflammation, and lung damage. Infection with influenza A viruses is associated with severe symptom exacerbations, which contribute to increased CF patient morbidity, bacterial colonization, and disease progression. While influenza infection rates do not differ between CF and non-CF patients, disease is more severe in those with CF. Human airway epithelial cells (HAECs) isolated from normal and CF patient donor lungs, were grown at an air-liquid interface to generate confluent, highly-differentiated monolayers of similar cellular composition to normal airways. HAEC cultures were then mock-infected or infected *in vitro* with influenza A/WSN/33 (H1N1) at a multiplicity-of-infection of 1. mRNA was isolated and analyzed by qRT-PCR. Following infection for 24 hours, expression of pro-inflammatory cytokines IFN- α , IL-6, IL-8, and TNF- α , was higher in CF than non-CF HAECs. CF HAECs also expressed higher levels of viral toll-like receptors TLR-3 and TLR-7. These findings show that the innate immune response of CF HAECs to *in vitro* influenza A virus infection is abnormal, which suggests that an excessive respiratory epithelial cell inflammatory response to infection contributes to the severity of symptom exacerbations in influenza-infected CF patients.

Keywords: Cystic Fibrosis, HAECs, symptom exacerbations, Influenza A (H1N1), TLRs

MIR-155 IMPACTS T CELL MIGRATION IN ACUTE GRAFT-VERSUS-HOST-DISEASE (AGVHD). N.C. Zitzer¹, P.A. Taylor², A. Nganheu¹, Y.A. Efebera¹, S.M. Devine¹, B.R. Blazar², R. Garzon¹, P. Ranganathan¹. ¹Comprehensive Cancer Center, The Ohio State University, Columbus, OH; ²Blood and Marrow Transplantation, Division of Pediatrics, Department of Medicine, University of Minnesota, Minneapolis, MN

We reported that miR-155 expression is upregulated in donor T cells during aGVHD and mice receiving miR-155 knock-out (KO) donor splenocytes do not exhibit lethal GVHD and have improved survival as compared to mice receiving wild type (WT) splenocytes. Here, we investigate the impact of miR-155 expression in T cell migration and elucidate the T cell population responsible for miR-155-mediated modulation of aGVHD. There was dramatic decrease in T cell infiltration of peripheral organs in recipients of miR-155-KO T cells as compared to WT T cells as evidenced by confocal microscopy of GFP-labeled donor cells. There was a significant decrease in chemokine receptor CCR5 mRNA and protein expression in miR-155-KO versus WT donor T cells in recipient mice with clinical aGVHD. Allo-activated miR-155 KO T cells show significantly reduced migration towards CCR5 ligands RANTES and MIP-1a using *in vitro* transwell migration assays, with an average migration index (MI) of 1.08, compared to the average MI of WT T cells of 4.52 ($p < 0.005$). We performed a B6 into F1 transplant using only CD4+ donor T cells. Median survival of recipients of WT CD4+ T cells ($n=13$) was 42 days, compared to 100% survival of recipient mice of miR-155 KO CD4+ T cells ($n=12$) on day 100, ($p < 0.0001$). Recipients of miR-155 KO CD4+ T cells also exhibited significantly lower aGVHD clinical ($p < 0.01$) and pathological scores ($p < 0.01$) than WT recipients. Our data suggest that miR-155 exerts its modulating effects in aGVHD by affecting T cell migration and indicates that the CD4+ T cells plays an important role in miR-155 regulation of aGVHD. Future experiments are underway to determine the role of miR-155 in regulatory and CD8+ T cell subsets in the modulation of aGVHD and evaluate the role of miR-155 in modulating T cell migration through other chemokine receptors.

Keywords: aGVHD, graft-versus-host-disease, T cell migration, microRNA-155

INHIBITION OF LUNG TISSUE NON-SPECIFIC ALKALINE PHOSPHATASE

ATTENUATES INFLUENZA-INDUCED ACUTE LUNG. P. S. Woods^{1,2}, L. Doolittle^{1,2}, and I. C. Davis¹ Department of Veterinary Biosciences¹, The Ohio State University
The Ohio State School of Medicine², The Ohio State University

Influenza A viruses are readily transmissible respiratory pathogens that remain a significant threat to human health. However, available vaccines and antiviral drugs have limited efficacy. Our limited understanding of influenza pathogenesis remains a major obstacle in improving influenza therapeutics. Extracellular nucleotides and nucleosides regulate fluid balance within the lung and can serve as leukocyte chemoattractants. We have previously shown that influenza infection of mice leads to increased ATP and adenosine accumulation in the airway lumen. Moreover, we demonstrated that A1-adenosine receptor activation contributes significantly to influenza-induced acute lung injury (ALI). Extracellular adenosine levels are regulated by cell surface enzymes that metabolize ATP to adenosine. Ecto-5'-nucleotidase (CD73) is a high-affinity, low-capacity enzyme that converts AMP to adenosine. It has been proposed that CD73 regulates extracellular adenosine concentrations under steady-state conditions within the lung. Tissue non-specific alkaline phosphatase (TNAP) is a low-affinity, high-capacity enzyme that catabolizes nucleotides in a non-specific manner. TNAP is therefore likely to play a more significant role in nucleotide breakdown in situations of robust nucleotide release, such as influenza infection. We found that influenza-induced ALI was not attenuated in CD73-knockout mice or by treatment of infected mice with a CD73 inhibitor (DPCPX). Hence, we hypothesized that TNAP mediates adenosine generation in influenza-infected mice and that inhibition of TNAP will attenuate influenza-induced ALI. To test our hypothesis, C57BL/6 mice were inoculated with 10,000 pfu/mouse of influenza A/WSN/33 (H1N1). Preliminary data suggest that influenza infection upregulates TNAP gene and protein expression and enzymatic activity as early as 2 days post infection (d.p.i.). Treatment of infected mice at 2 and 4 d.p.i. with 50µl of 10µM TNAP inhibitor or vehicle (DMSO) intranasally significantly attenuated hypoxemia, pulmonary edema, and immune cell infiltration. These data suggest that TNAP inhibition attenuates influenza-induced ALI, most likely by reducing inflammation and fluid accumulation within the lung.

Key Words: Influenza, Acute Lung Injury, Nucleotide Signaling

MOLECULAR AND CELLULAR BIOLOGY

IDENTIFYING THE ROLE OF NOVEL TAX-1 INTERACTING PROTEIN SNX27 IN HTLV-1 INFECTION. Jacob Al-Saleem^{1,2,3}, Nikoloz Shkriabai^{1,4}, Mamuka

Kvaratskhelia^{1,4}, Lee Ratner⁶, and Patrick L. Green^{1,2,3,4}

¹Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA; ²Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA; ³College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA; ⁴Department of Pharmaceutics and Pharmaceutical Chemistry, The Ohio State University, Columbus, OH, USA; ⁵Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University, Columbus, OH, USA; ⁶Division of Oncology, Washington University, St Louis, MO, USA

Human T-cell Leukemia Virus Type-1 (HTLV-1) is a complex retrovirus infecting 15-20 million people worldwide, and is the etiological agent of an aggressive malignancy of CD4+ T-cells termed Adult T-Cell Leukemia. By contrast, HTLV-2 is non-pathogenic in humans. Both HTLV-1 and HTLV-2 express related Tax proteins termed Tax-1 and Tax-2, respectively. Studies have revealed that Tax-1 contains a C-terminal post synaptic density protein (PDZ) binding motif (PBM) which is absent in Tax-2. We aim to determine whether this domain in Tax-1 is required for protein-protein interactions. These could prove crucial for differences in pathogenesis of the two viruses. Using Tax-1 mutants lacking the PBM we identified several candidates via a mass spectrometry based proteomic screen. One candidate was Sorting Nexin 27 (SNX27), a member of the sorting nexin family of proteins which are involved in endocytosis and protein trafficking. SNX27 is a unique member of the sorting nexin family in that it contains a PDZ domain. Published literature has shown that SNX27 is involved in GLUT1 recycling from lysosomes to the plasma membrane, and without SNX27 GLUT1 is internalized and degraded. We propose that Tax-1 interaction with SNX27 may alter this SNX27 regulation of GLUT1, which is the receptor molecule for HTLV-1. This modulation could prove beneficial to HTLV-1 since plasma membrane bound receptor molecules have been shown to interfere with virion release and infectivity in other retroviruses, including HIV. We confirmed that Tax-1 interacts with SNX27 through the PDZ. We also demonstrated that SNX27 overexpression does not affect Tax-1 transactivation, but resulted in decreased p19 release into the supernatant. Our future work will determine the mechanistic role of the SNX27 and Tax-1 interaction in modulation of GLUT1. These studies will further our knowledge on HTLV-1 virus release and infectivity, and may potentially lead to new therapies to prevent infection.

Keywords: HTLV-1, ATL, SNX27, GLUT1

SALMON POISONING DISEASE: CANINE IMMUNE RECOGNITION OF *NEORICKETTSIA HELMINTHOECA*. K. Bachman, M. Lin, Y. Rikihisa.

Department of Veterinary Biosciences, College of Veterinary Medicine,
The Ohio State University

Neorickettsia helminthoeca, the causative agent of Salmon Poisoning Disease (SPD), is an obligate helminth-borne intracellular bacteria that infects reticuloendothelial cells of wild and domestic canids. Endemic to the Pacific Coast, dogs acquire SPD by ingesting salmonid fish infested with *N. helminthoeca*-infected trematode. The current definitive diagnostic test - PCR, takes time and requires trained personnel and special equipment. Due to the high mortality rate of SPD (>90%), there is a need for a more rapid serodiagnostic test. We have previously identified, cloned, and purified 5 antigenic, surface exposed, recombinant outer membrane proteins (OMPs) of *N. helminthoeca*: P51, NSP-1/2/3, and SSA. The aim of this study was to determine (1) if these recombinant OMPs will be detected by experimentally infected and clinical SPD sera, and (2) which of these recombinant OMPs, if any, will react most strongly. Preliminary Western blotting data using both experimental and naturally infected dog sera showed seroreactivity to P51, SSA, NSP1, and NSP2 but not NSP3. Both sera reacted most strongly to SSA, followed by NSP2. Future research will focus on obtaining more clinical SPD samples to perform Western blotting to support or refute this preliminary data. Furthermore, highly antigenic, surface exposed oligopeptide domains within these *N. helminthoeca* OMPs will be determined by ELISA to examine the sensitivity and specificity compared to other closely related members like *N. risticii* (the agent of Potomac Horse Fever) and *N. sennetsu* (the agent of Sennetsu Ehrlichiosis in humans). Applications of this research include development of a more rapid, sensitive, and specific immunodiagnostic test and of a vaccine for SPD.

Keywords: *Neorickettsia helminthoeca*, Salmon Poisoning Disease, Diagnosis, Molecule cloning, Bacterial Outer Membrane Proteins, Recombinant Proteins, Trematode, Western Blotting

IMPROVING EFFICIENCY IN SAUGEYE PRODUCTION USING CRYOPRESERVED MILT. B.Blawut¹, M. Krcmarik¹, B. Wolfe³, M.C. da Silva², R. D. Zweifel⁴, D. Sweet⁴, & S.Hale⁴

1 Ohio State University, College of Veterinary Medicine, Department of Veterinary Preventive Medicine, 1920 Coffee Rd. Columbus, OH 43210

2. Ohio State University, College of Veterinary Medicine, Department of Veterinary Clinical Sciences, 601 Vernon Sharp St. Columbus, Ohio 43210

3 Morris Animal Foundation, 720 S. Colorado Blvd., Suite 174A Denver, CO 80246

4 Ohio Division of Natural Resources, 2045 Morse Rd Service Road, Columbus, OH 43229

Periodically, saugeye (*Sander canadensis* x *S. vitreus*) production goals have been compromised by weather-related alterations in broodstock collection seasons. Sperm cryopreservation can be used to store excess milt for long term use. Recently, hypertonic extenders have been shown to increase post-thaw motility, viability and fertility of ram (*Ovis aries*) sperm. The objective of this study was to assess the effect of three different extenders on cryopreserved sauger milt post-thaw motility and viability. Ejaculates from twenty males were divided into three aliquots and diluted in Rathbun (Moore 1987) extender at different osmolarities (350, 500, or 750 mosm/kg) to one billion sperm per milliliter. Samples were then diluted 1:1 with 10% dimethyl sulfoxide, distributed into 0.25 ml straws and cooled in liquid nitrogen (LN2) vapor for ten minutes prior to submersion in LN2. After cryopreservation, straws were thawed in a 21°C water bath for 30 seconds and post-thaw motility and plasma membrane integrity were assessed. Post-thaw motility and viability were significantly different ($p < 0.05$) in samples cryopreserved at different osmolalities. These methods will be further developed for scaling of cryopreservation protocols to provide fry production security during unfavorable weather conditions.

Keywords: Cryopreservation, Sperm, Wildlife

THE EFFECT OF TRYPAN BLUE ON POSTERIOR CAPSULE OPACIFICATION IN AN EX VIVO CANINE MODEL BM Brash, DA Wilkie, AJ Gemensky-Metzler, HL Chandler, Department of Veterinary Clinical Sciences, The Ohio State University

Purpose. To determine if trypan blue (TB) reduces lens epithelial (LEC) or corneal endothelial cell viability. **Methods.** Tissue was harvested from canine cadavers. Cultured LECs were treated with TB at 0, 0.05, 0.1, 0.2, or 0.3% for 30, 60, or 120 seconds. Cell morphology was evaluated and an LDH viability assay performed. Cultured LECs were treated with 0 and 0.3% TB for 120 seconds and an apoptosis assay was performed to assess caspase-3 activity. To evaluate the effects of TB on *ex vivo* PCO, following mock cataract surgery, lens capsules were treated with 0 and 0.3% TB at the above times and maintained in culture for two weeks. Capsules were monitored for changes in cell density and morphology; histology was performed at experimental completion. Corneal endothelial cells were treated with 0 and 0.3% TB for 120 seconds and an LDH viability assay performed. **Results.** TB did not significantly reduce LEC density. While TB-treated LECs demonstrate higher rates of cell death compared to vehicle control, the difference was not significant. Induction of apoptotic signaling was found in TB-treated LEC cultures. *Ex vivo* PCO formation was not significantly different in any treatment group. Endothelial cells treated with TB or vehicle showed no significant differences in cell death. **Conclusions.** TB induced low levels of LEC death via apoptotic signaling cascades but was not effective at reducing *ex vivo* PCO formation. TB did not induce endothelial cell death. Funded by ACVO Vision for Animals Foundation grant (VAF2014-01). Trypan blue provided by Acrivet.

Keywords: canine, lens, cataract surgery, posterior capsule opacification, trypan blue

ANTITUMOR ACTIVITY OF SELECTED CHEMOTHERAPY AGENTS ON A FELINE BRONCHIOLOALVEOLAR LUNG CARCINOMA CELL LINE. D. L. Burroughs, S. Roy, and G. Lorch. Department of Veterinary Clinical Sciences.

The prognosis for feline advanced lung cancer remains poor and new treatments are needed. Cell culture models provide a framework for identification of the most important biochemical pathways involved in tumorigenesis. Evaluation of gene and protein expression levels in biospecimens aids in the identification of molecular targets expressed in the tumor. Aberrant signaling of epidermal growth factor receptor (ERBB or EGFR) and its family members are known to be overexpressed in human non-small cell lung cancer. We hypothesized that EGFR gene amplification and protein overexpression would be present in feline bronchioloalveolar lung carcinoma (BAC). The objectives of this study were to investigate the biologic activity of cytotoxic chemotherapeutics and EGFR small molecule inhibitors on a feline BAC cell line, and evaluate EGFR gene expression and protein levels in primary BACs and associated digit metastases from two cats. Relative viability and the half-maximal inhibitory concentrations (IC_{50}) were determined after 72 hr exposure to vinorelbine, docetaxel, paclitaxel and EGFR inhibitors, sapitinib and poziotinib. With respect to currently used cytotoxic chemotherapeutics, treatment with paclitaxel achieved the lowest IC_{50} (6.9 nM). Quantitative real-time PCR identified EGFR, EGFRvar1 and HER2 mRNA expression in all primary tumors and digit metastases. mRNA expression levels of ERBB members differed between primary lung tumors and digit tumors. Determination and quantification of tumor ERBB protein levels are being optimized. Differing expression levels of ERBB members in primary tumors vs. metastases suggests a pan-EGFR inhibitor might offer an effective therapeutic opportunity to manage the feline lung cancer patient.

Keywords: Feline, Lung, Cancer, Chemotherapy

CANINE MODEL OF PROSTATE CANCER AND THE ROLE OF THE GASTRIN-RELEASING PEPTIDE RECEPTOR (GRPR). R. Y. Camiener, S. M. Elshafae, W. P. Dirksen, and T. J. Rosol, Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.

Spontaneous prostate cancer occurs in only two species: humans and dogs. The cancer has similar characteristics in both species, and canine prostate cancer can serve as an effective model for developing novel diagnostic tests and therapy. In early-stage prostate cancer (PrC), the GRPR is overexpressed, which makes GRPR a desirable molecular target. We have shown that human GRPR signaling promoted prostate cancer cell proliferation and enhanced their migration and invasion *in vitro*. Additionally, bombesin, a GRPR agonist, increased the growth of canine prostate cancer cells (Ace-1) in nude mice, forming larger tumors. However, the effects of canine GRPR and its activity in canine prostate cancer cells are not yet known. Using quantitative RT-PCR we will measure GRPR mRNA expression in canine PrC cell lines (n=5) and spontaneous tumors (n=4). We found that a subset of spontaneous canine prostate cancers had high levels of GRPR. Concurrently, we cloned canine GRPR cDNA and stably transduced an expression vector of canine GRPR into the canine prostate cancer cell, Ace-1. Our lab developed Ace-1 cells from a primary prostatic carcinoma of an eight-year-old male castrated Labrador retriever. The Ace-1 cells can be successfully transplanted to the prostate glands of cyclosporine (CYA)-treated dogs to form focal tumors. We transduced human GRPR into Ace-1 cells and injected them into canine subjects, but found that CYA-treated dogs rejected the cells. We expect to avoid an intense immunological reaction in canine subjects by using Ace-1 cells transduced with canine GRPR. This will enable us to enhance the canine PrC model to better understand the disease and develop improved diagnostics and treatments.

Keywords: Prostate, Cancer, GRPR, Canine

TOPOGRAPHY INFLUENCES GLIAL AND NEURONAL MIGRATION UNDER INFLUENCE OF LAMININ IN VITRO. J Cronin^{1,2}; C Czeisler, PhD^{1,3}; A Short, PhD^{1,4}; J Winter, PhD^{1,4}; JJ Otero, MD, PhD^{1,3}

¹The Ohio State University, ²College of Veterinary Medicine, ³College of Medicine, ⁴College of Engineering

The interaction between topography and chemical substrates within the CNS has been poorly characterized with regard to CNS migration, an essential function in embryonic brain development and pathologically migrating gliomas across species. We sought to determine the contribution of scaffold topography to the migration of CNS cells by modeling different scaffolds in vitro. We mimicked two types of CNS scaffolds encountered by neural stem cells during development by constructing different diameter electrospun polycaprolactone (PCL) fiber mats, a substrate that is topographically similar to aligned brain scaffolds. We compared the effects of 10um fibers (large diameter fibers made to mimic blood vessels) with those of 800nm diameter fibers (small diameter fibers made to mimic radial glial processes) on the migration of glia and neurons. We demonstrate that glial and neuronal migration on 10um fibers is improved by laminin coating. Without laminin coating, little to no cell migration occurs on the 10um fibers. By contrast, 800nm fibers induce some degree of glial and neuronal migration without laminin, and this effect is potentiated by the presence of laminin. We also show that neurons on 10um fibers follow the migration of glia, while neurons on 800nm fibers are capable of migrating independently of glia. We propose that the physical structure of distinct scaffolds, in combination with key chemical substrates such as laminin, induces unique signaling cascades that regulate the migration of glia and neurons distinctly. These findings may have broad implications to understanding the response of CNS cells to combined topographical and chemical cues.

Keywords: CNS migration, Topography, Laminin, Embryonic brain development, Pathologically migrating gliomas

ANALYSIS OF FAS-MEDIATED APOPTOSIS IN CANINE CERVICAL SPONDYLOMYELOPATHY. E. Curtis, R.C. da Costa. Department of Veterinary Clinical Sciences

Cervical spondylomyelopathy (CSM), or Wobbler Syndrome, is a common neurological disease affecting the cervical spine of large and giant breed dogs. Despite the multitude of surgical and conservative treatment options, the long-term survival of CSM-affected dogs remains unchanged. The reasons for this short-term survival are not known but may involve adjacent segment disease and apoptosis. The close proximity of the spinal cord with cerebrospinal fluid (CSF) allows us to look for biomarkers to better understand the apoptotic mechanism. We know that detection is possible via the CSF from previous work in our laboratory. Evidence in both human and animal models suggest involvement of Fas-mediated apoptosis, which led us to a working hypothesis that pro-apoptotic biomarkers caspase-3 and soluble Fas will be increased in the CSF of CSM-affected dogs compared to dogs without CSM, and will correlate with severity of neurological deficits and spinal cord compression. CSF was collected from 24 Great Dane dogs, 13 CSM-affected dogs (confirmed with magnetic resonance imaging) and 11 control dogs. A canine-specific solid-phase sandwich ELISA was used for the quantitative determination of canine caspase-3 and soluble Fas in the CSF samples. Based on previous studies in rodents and people with traumatic brain injuries, both caspase-3 and soluble Fas were expected to have significantly increased concentrations in the CSF of CSM-affected dogs affected by CSM as compared to the control group. Neither assay showed statistically significant differences between the CSM-affected dogs and the control dogs. The lack of results could indicate a true negative result, or it may be a consequence of poor sensitivity of the assays. Discovering the detailed mechanism of apoptosis is extremely important for finding a successful treatment. Once the exact pathogenesis is discovered, the use of anti-apoptotic drugs can be evaluated in the treatment of dogs with CSM.

Keywords: Cervical Spondylomyelopathy, Wobbler Syndrome, Apoptosis, Spinal Cord

THE EFFECT OF HYPOTHERMIA ON INFLAMMATORY AND GROWTH FACTOR SIGNALING PATHWAYS IN ACUTE LAMINITIS. K. Dern, M. Watts, A. van Eps, J. Belknap. Dept of Veterinary Clinical Sciences

Recent work in sepsis-related laminitis (SRL) has revealed that not only does an initial injury result in similar cytoskeletal and structural changes in the laminar epithelium as reported for equine metabolic syndrome associated laminitis (EMSAL), but that the only therapy proven to mitigate laminar damage in SRL is continuous digital hypothermia (CDH). Although early reports indicated that CDH is effective via inhibition of inflammatory signaling, we have not found this effect of CDH in late stage laminitis. Due to recent work demonstrating insulin-induced activation of laminar mTORC1/RPS6 signaling (characteristic of growth factor-related signaling) in EMSAL, we hypothesized that the same signaling is induced by STAT3 activation in SRL and that CDH blocks this signaling. We assessed laminar concentrations of activated/phosphorylated proteins of interest in Standardbred horses (n=16) administered either oligofructose (n=8) or water (n=8) in the OF model of SRL, with one forelimb maintained at ambient temperature and one limb placed in ice water (CDH) for 24h starting 12 h after OF/water administration. Laminar tissue was harvested from both forelimbs 24h after initiation of CDH. Immunoblotting revealed increased ($P<0.05$) lamellar concentrations of phosphorylated/activate forms of STAT3, p70S6K, and RPS6 in the ambient limb; CDH inhibited the activation of p70S6K and RPS6. Real time quantitative PCR assessment of laminar mRNA concentrations of pro-inflammatory cytokines, chemokines, endothelial adhesion molecules, and COX-2 revealed that CDH did not significantly decrease lamellar mRNA concentration of any of the assessed inflammatory mediators. These results confirm that similar mTORC1-related signaling occurs in the laminae in SRL as previously reported for EMSAL, and establishes an association between the protection conferred by CDH and a profound decrease in the activation of the growth factor-related signaling proteins, mTORC1 and RPS6.

Keywords: laminitis, hypothermia, mTORC1, RPS6, inflammation, sepsis-related laminitis

TRACKING TRANSCRIPTOME MODIFICATIONS RESPONSIVE TO THE ESTROUS CYCLE IN THE MOUSE UTERUS. A Diedrich, C Koivisto, G Leone. College of Veterinary Medicine (Diedrich, Koivisto), School of Biological Sciences-Molecular Virology, Immunology and Molecular Genetics, College of Medicine (Leone) The Ohio State University

Endometrial cancer is the most common malignancy of the female reproductive tract. Between 80-85% are classified as type I endometrial carcinoma (EMC). One known risk factor for EMC is the persistent exposure to estrogen. We have created a reproducible mouse model of EMC (100% incidence of disease) by conditionally deleting the tumor suppressor protein Pten specifically in uterine epithelium. Based on this, we hypothesize that 1) Pten exerts its tumor suppressor effects in EMC by regulating multiple sex hormone signaling pathways and 2) hormonal changes during the normal estrous cycle plays a significant role in Pten function. To address these questions, we are collecting whole transcriptome data from wild-type FVB mice from each estrous cycle stage. This will be used to evaluate which pathways associated with Pten are responsive to hormonal signaling. These pathways will then be evaluated in heterozygous knockout Pten mice to elucidate, or rule out, their potential role(s) in the progression of type 1 EMC. We expect to find that the transcriptome of the endometrium is highly variable between stages of the estrous cycle. Along with the goal of describing the progression of type 1 EMC, we expect this data will demonstrate the critical importance of the reproductive cycle's fluctuations in the progression of other cancers.

Keywords: Endometrial Carcinoma, Pten, Estrous Cycle

THE EFFECT OF HDACI (AR-42) ON CANINE PROSTATE CANCER METASTASIS. S. Elshafae¹, N. Kohart¹, L. Altstadt¹, W. Dirksen¹ and T. Rosol¹. Department of Veterinary Biosciences¹, The Ohio State University, Columbus, OH, USA.

Canine prostate cancer (PCa) is an excellent preclinical model for human PCa. AR-42 is a novel histone deacetylase inhibitor (HDACi) developed at Ohio State University that inhibits proliferation of multiple myeloma and lung and hepatocellular cancer. We investigated whether AR-42 would prevent or decrease metastasis of PCa to bone. We measured the proliferation, cell viability, invasion, and metastasis of a canine prostate cancer cell line (Ace-1) after treatment with AR-42, and measured the expression of EMT, stem cell-related markers and anoikis resistance genes in Ace-1-treated cells. We investigated the efficacy of AR-42 to prevention PCa metastases in nude mice injected in the left cardiac ventricle with Ace-1 cells. The results showed that AR-42 inhibited proliferation of Ace-1 cells in a time and dose-dependent manner. The IC₅₀ concentration of AR-42 for Ace-1 cells was 0.42 μ M after 24 hr of treatment. AR-42 induced apoptosis and decreased the migration potential and stem cell properties of Ace-1 cells in vitro. AR-42 downregulated E-cadherin, N-cadherin, TWIST, Myoferlin, and anoikis resistance and osteomimicry genes while it upregulated Snail, PTEN, FAK and ZEB1 in Ace-1 cells. Interestingly, AR-42 decreased the number of bone metastases in nude mice and induced apoptosis and morphological changes in the metastases. These data demonstrated that AR-42 decreased the progression of PCa bone metastasis, induced apoptosis in cancer cells established in bone and diminished the effect of PCa cells on bone cells by downregulation of osteomimicry genes. Future studies will evaluate the effect of AR-42 on the PCa bone microenvironment.

Keywords; Prostate cancer, AR-42, HDACi, Metastasis

INSULIN-RELATED GROWTH FACTOR SIGNALING EVENTS IN THE EQUINE LAMINAE USING A MODEL OF EQUINE METABOLIC SYNDROME. O. Hegedus, M. Watts, P. Weber, K. Woltman, J. Belknap. Dept. of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University, Columbus, OH (Hegedus, Watts, Balknap). Dept. of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI (Weber and Woltman).

Equine metabolic syndrome associated laminitis (EMSAL) is the most common presentation of laminitis in equine practice with limited pharmaceutical options for treatment. Equine metabolic syndrome (EMS) is defined by a set of physical and pathophysiologic traits, which include regional or general adiposity and insulin dysregulation. A recently established model of EMSAL is a euglycemic hyperinsulinemic clamp (EHC) model which results in manifestations of laminitis (both clinical and histopathologic laminar changes) in approximately 48 hours. We hypothesized that insulin activates RPS6 (a protein associated with epithelial cell dysregulation in human cancers) via activation of growth factor-related signaling pathways MEK/ERK and PI3K/Akt/mTORC1, resulting in dysregulation of the laminar basal epithelial cells and thus laminar failure. In the current study, 16 healthy Standardbred horses were randomly placed either 1) on the protocol previously reported to induce laminitis in which a EHC was instituted for 48 H (or until signs of lameness, n=8), or 2) administered saline for 48 H (CON, n=8). Upon Western blot analysis of laminar samples, EHC samples have significantly increased ($p<0.05$) concentrations of phosphorylated forms of RPS6 at both Ser 240/244 and Ser 235/236 moieties (vs. controls). Additionally, EHC samples also have increased ($p<0.05$) concentrations of phosphorylated forms of signaling proteins upstream of RPS6 including p70S6K, p90RSK, Akt, and ERK 1/2. These findings provide a basis for the evaluation of potential therapeutic avenues to control signaling pathways resulting in the activation of RPS6 in an effort to prevent progression to laminar failure in horses with EMS

Keywords: Equine metabolic syndrome, laminitis, ribosomal protein S6, insulin

DOWNREGULATION OF SAMHD1 EXPRESSION CORRELATES WITH INCREASED MICRORNA-181 LEVELS IN SÉZARY SYNDROME PATIENT CD4+ T-CELLS. R. Kohnken¹, K.M. Kodigepalli¹, A. Mishra^{2,3}, P. Porcu^{2,3,4} and L. Wu^{1,2,5}

¹Center of Retrovirus Research, Department of Veterinary Biosciences; ²Comprehensive Cancer Center; ³Division of Hematology; ⁴Department of Internal Medicine; ⁵Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, 43210, USA

Sézary syndrome (SS) is a rare subtype of human cutaneous T-cell lymphoma (CTCL) that is characterized by aggressive spread of neoplastic CD4+ T-cells from the skin into the bloodstream with metastasis to visceral organs. The deoxynucleoside triphosphohydrolase SAMHD1 is highly expressed in normal human CD4+ T-cells, while its expression is down-regulated in CD4+ T-cells from SS patients. MicroRNA (miR) dysregulation is an important epigenetic mechanism in the pathogenesis and progression of SS. MiR-181 has been shown to inhibit SAMHD1 expression in human lymphoma and leukemia cell lines and was recently identified as an important prognostic biomarker in CTCL. However, whether SAMHD1 is down-regulated by miR-181 in primary neoplastic CD4+ T-cells from SS patients is unknown. Compared to normal CD4+ T-cells, SAMHD1 protein expression is significantly reduced in CD4+ T-cell lines derived from lymphoma or leukemia patients and primary CD4+ T-cells from SS patients, which inversely correlates with increased miR-181 levels in these cells. Over-expression of miR-181b in primary CD4+ T-cells from healthy donors significantly decreased SAMHD1 protein level, but not mRNA level. In contrast, inhibition of miR-181b in a CD4+ T-cell line increased the level of SAMHD1 protein expression. Our results demonstrate that miR-181 is an important regulator of SAMHD1 protein expression in neoplastic CD4+ T-cells, likely through a mechanism of translational inhibition.

Keywords: Sèzary Syndrome, SAMHD1, microRNA

INDUCIBLE CRE-MEDIATED ABLATION OF E2F7 AND E2F8 IN THE MOUSE SMALL INTESTINE. M. Maglaty¹, M. Cuitino², J. Rakijas², and G. Leone². ¹College of Veterinary Medicine; ²Department of Molecular Virology, Immunology, and Medical Genetics, College of Medicine, The Ohio State University.

The E2fs are transcription factors that act as master regulators of cell proliferation. *In vivo* studies have shown that the atypical repressors E2f7 and E2f8 play a crucial role in the control of proliferation and apoptosis during embryonic development; their ablation leads to embryonic death by E11.5. Further studies have demonstrated their involvement in the regulation of endocycles in trophoblast cells and hepatocytes. However, their role in other tissues and the molecular pathways they regulate remain unknown. To explore how the atypical E2f repressors control proliferation *in vivo*, an inducible intestinal-specific cre transgene (Ah-cre) and conditional knockout alleles of E2f7/8 generated in the Leone lab were used. We hypothesized that ablation of E2f7/8 in the small intestine could lead to altered proliferation and disruption of the tissue architecture and function. Two-month old mice were administered β -naphthoflavone to induce Ah-cre expression and harvested 4 and 14 days later. Duodenum and jejunum were collected for histology and immunostaining of proliferation and apoptosis markers. The deletion of E2f7/8 was confirmed by PCR genotyping of DNA from isolated duodenal epithelium. No changes in intestinal histology were observed. Markers of DNA synthesis, mitosis and apoptosis are being used to quantify changes in the cell cycle and cell death. Future directions include mRNA isolation from intestinal epithelium to look for changes in expression of E2F7/8 transcriptional targets by RT-qPCR. Gaining insight into the normal functions of E2f7 and E2f8 *in vivo* will contribute to a better understanding of their implications in the pathogenesis of human disease.

Keywords: E2F7, E2F8, apoptosis, proliferation, embryonic development, cell cycle control, transcription factors, cre transgene, conditional knockout

PLATFORM PRESENTATION

PRMT5 IS UPREGULATED IN HTLV-1-MEDIATED T-CELL TRANSFORMATION AND SELECTIVE INHIBITION ALTERS VIRAL GENE EXPRESSION AND INFECTED CELL SURVIVAL. A. Panfil, J. Al-Saleem, C. Howard, J. Mates, J. Kwiek, R. Baiocchi, and P. Green. Depts. of Veterinary Biosciences

Human T-cell leukemia virus type-1 (HTLV-1) is a tumorigenic retrovirus responsible for development of adult T-cell leukemia/lymphoma (ATLL). This disease manifests after a long clinical latency period of up to 2-3 decades. Two viral gene products, Tax and HBZ, have transforming properties and play a role in the pathogenic process. Genetic and epigenetic cellular changes also occur in HTLV-1-infected cells, which contribute to transformation and disease development. However, the role of cellular factors in transformation is not completely understood. Herein, we examined the role of protein arginine methyltransferase 5 (PRMT5) on HTLV-1-mediated cellular transformation and viral gene expression. We found PRMT5 expression was upregulated during HTLV-1-mediated T-cell transformation, as well as in established lymphocytic leukemia/lymphoma cell lines and ATLL patient PBMCs. shRNA-mediated reduction in PRMT5 protein levels or its inhibition by a small molecule inhibitor (PRMT5i) in HTLV-1-infected lymphocytes resulted in increased viral gene expression and decreased cellular proliferation. PRMT5i also had selective toxicity in HTLV-1-transformed T-cells. Finally, we demonstrated that PRMT5 and the HTLV-1 p30 protein had an additive inhibitory effect on HTLV-1 gene expression. Our study provides evidence for PRMT5 as a host cell factor important in HTLV-1-mediated T-cell transformation, and a potential target for ATLL treatment.

Keywords: ATLL; HBZ; HTLV-1; PRMT5; Tax; lymphoma; transformation

CHARACTERIZATION OF LIVING SYNOVIAL EXTRACELLULAR MATRIX SCAFFOLDS FOR GENE DELIVERY. N. Reisbig, H. Hussein, E. Pinnell, A. Bertone. Department of Veterinary Clinical Sciences. College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.

Cartilage injury and degeneration is a leading cause of disability in humans and horses. There is currently no treatment to reverse the loss of chondrocyte function. However, regenerative cells, including synovial mesenchymal stromal cells (SMSCs) and anabolic agents, combined with scaffolds for targeted delivery is a promising option. The synovium offers the advantage of containing highly metabolic cells that readily proliferate and have been shown to secrete joint restorative substances. Synovial cells can also be genetically engineered to secrete growth factors. Our hypothesis was that decellularized synovium (synECM) seeded with synoviocytes genetically modified with Bone morphogenetic protein 2 (BMP2-SMSCs) would show enhanced scaffold migration, engraftment, and living cell counts while producing significant levels of BMP-2. Synovium from equine stifles was harvested and decellularized. Synoviocytes, from digested synovium, were either transduced with Ad-BMP-2/Green fluorescence protein (SMSCs-BMP2/GFP) for tracking and documentation of gene delivery potential of the living scaffolds, or left as control cells (SMSCs). The synECM was seeded and incubated using a 30% fetal bovine serum gradient. The explants were examined for cell growth, CD-90 expression, viability, and morphology on day 3, 7 and 14, and the supernatant were analyzed for BMP-2, hyaluronic acid (HA) and proteoglycan (PG) secretions. Increased cell counts, cell migration into the scaffold and evidence of cell differentiation support that synECM seeded with SMSCs or BMP2-SMSCs produced a living synovial scaffold. Significant levels of BMP-2 concentrations were produced by the BMP2/GFP-SMSCs, followed by an increase in both HA and PG indicating gene production and enhanced synovial function. The results of this study suggest that synECM seeded with normal and genetically altered SMSCs may have potential for treatment of cartilage injuries.

Keywords: synovium, Bone morphogenetic protein 2, cartilage, synoviocytes, extracellular matrix scaffold.

MICRORNA AS A HOST DETERMINANT OF SEVERITY IN INFLUENZA A VIRUS INFECTION. L.D. Schermerhorn*, P. Woods*, S.P. Nana-Sinkam[&], I.C. Davis*

*Department of Veterinary Biosciences, The Ohio State College of Veterinary Medicine

[&]Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, The Ohio State University College of Medicine

As the 8th leading cause of attributable annual mortality in the USA, influenza A viruses are a significant public health concern. Severe primary influenza can result in acute lung injury (ALI), which is characterized by hypoxemia, pulmonary edema, and impaired lung function. Currently, there are few therapeutic options for patients with influenza A virus-induced ALI, especially with onset of acute respiratory distress syndrome (ARDS). MicroRNAs (miRs) are short, endogenous, non-coding RNA's that regulate expression of multiple genes simultaneously at the post-transcriptional level. miRs regulate host immunity and cell survival, and altered miR expression may contribute to the pathogenesis of other forms of ALI. Influenza A virus infection has been shown to alter miR expression at the whole lung level. We infected C57BL/6-congenic *adora1*-KO and F508del CFTR-heterozygous mice with 10,000 pfu/mouse influenza A/WSN/33 (H1N1) virus, and performed primary isolation of alveolar type-2 epithelial cells (ATII cells) as described previously. Total RNA isolation with preservation of small RNA's (<25 nucleotides) was performed. Using probes specific for *miR-155-5p*, we quantified ATII cell derived *miR-155-5p* expression in our experimental groups, finding strain-specific differences in expression and other clinical parameters. Other studies in our lab show *miR-155* induction is STAT-1 dependent, and an overall reduction of inflammatory phenotype manifests in global *miR-155*-knockout (155-KO^G) mice in response to infection. Further work indicates ARDS attenuation is dependent on 155-KO^G stromal cells, not myeloid cells, suggesting specific ATII cell dependency for the pathogenesis of ALI and ARDS. Furthermore, novel experiments in selective antagonism and inhibition of ATII cell STAT-1 phosphorylation have resulted in subsequent improvement in clinical prognostic parameters that are analogous to those used in human patients. These findings strongly suggest that *miR-155* is a previously unidentified host determinant of influenza severity, and is a valuable target for therapeutic development and intervention.

Keywords: Influenza, microRNA, acute lung injury, ARDS, qRT-PCR

WNT SIGNALING IN PROSTATE CANCER GROWTH AND BONE METASTASIS.

W. Supsavhad, W. Dirksen, S. Elshafae, B. Hassan, N. Kohart, L. Altstadt and T. Rosol.
Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University

Prostate cancer is the second most common cancer in men worldwide and fatal bone metastasis occurs in 17% of patients. Ace-1-Dkk-1, a canine prostate cancer overexpressing human Dkk-1 was used in this study to investigate whether enhanced Wnt/JNK signaling could alter tumor growth, metastasis and the bone microenvironment. Evidence has shown in prostate cancer that Dkk-1 up-regulated the non-canonical Wnt/JNK pathway resulting in downstream alterations in gene expression important in bone formation, cell proliferation and epithelial to mesenchymal transformation (EMT) of tumor cells. Inhibition of non-canonical Wnt/JNK signaling using a JNK inhibitor (SP600125) significantly increased the mRNA expression of genes that induce bone formation as well as decreased bone lysis in vitro. Dkk-1 increased tumor volume in mice. When mice were injected subcutaneously with Ace-1-Dkk1, treatment with SP600125 significantly reduced tumor size and altered tumor cell morphology. However, treatment with SP600125 did not alter tumor size in mice that were injected intra-tibially with Ace-1-DKK1 cells. Inhibiting non-canonical Wnt/JNK signaling using SP600125 resulted in decreased tumor volume but did not alter tumor size in bone.

Keywords: Wnt signaling, prostate cancer, bone metastasis

ROLE OF STAT3 IN PROSTATE INVOLUTION AND CANCER CELL DEATH

KA Zabrecky¹, BW Simons², and EM Schaffer²

¹The Ohio State University CVM, Columbus, OH; ²The Brady Urological Institute, Johns Hopkins University, Baltimore, MD

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that, when activated by phosphorylation, is involved in a variety of pathways including proliferation and survival. In normal epithelial cells, STAT3 induces a pro-apoptotic response. This is also true for the mammary gland, a hormone dependent gland that involutes after the loss of hormonal signaling after lactation which displayed delayed involution in a STAT3 knockdown mouse model. Previous studies in our laboratory have shown STAT3 signaling in the prostate, another hormone dependent gland, after androgen withdrawal or castration. In prostate cancer, the common treatment involves anti-androgen drug therapy to induce involution of the gland and cancer cells. Interestingly, the role of STAT3 in prostate cancer cells is anti-apoptotic and prolongs the cancer's survival. Thus, we hypothesized that STAT3 knockout prostates would have delayed involution and decreased apoptotic markers after castration while STAT3 knockdown cancer cell lines would have increased apoptosis after androgen withdrawal. To investigate the role of STAT3 in normal prostate involution, PBCre STAT3 F/F knockout and B6 wild type (WT) control mice were castrated and harvested at either 4 or 6 days. The prostates were weighed and analyzed by immunohistochemistry and western blot for P-STAT3 and apoptotic markers. To investigate STAT3 in prostate cancer, 2 cell lines, LNCAP and LAPC4 with shRNA knockdowns for STAT3, were subject to androgen withdrawal with the growth/death measured and western blot preformed for apoptotic markers. It is important to understand the role of STAT3 in normal and cancer tissue and optimize biomedical research models to understand this potential target in cancer cell therapy.

Keywords: STAT3, castration, prostate cancer, apoptosis, androgens

STRUCTURE/FUNCTION

INTRAVENOUS ADMINISTRATION OF COBALT CHLORIDE IS ASSOCIATED WITH HEMODYNAMIC ALTERATIONS IN HORSES. Burns TA, Dembek K, Kamr A, Dooley B, Dunbar LK, Brewington S, Aarnes TK, Bednarski KS, O'Brien C, Lakritz J, Toribio RE.

Cobalt is a substance of abuse in humans and animals performing in strenuous athletic competitions. When administered at pharmacologic doses, it has been associated with induction of erythropoietin release, increased hematopoiesis, which is thought to confer competitive advantage. Cobalt chloride (CoCl₂) is reportedly given to racehorses to enhance performance, and recently, allowable limits for cobalt have been set by several racing jurisdictions for post-race illicit substance testing of blood and urine. While preliminary single-dose pharmacokinetic data have been published for CoCl₂ in horses, information regarding the effects of repeated dosing (which is how the substance is reportedly used illicitly in performance horses) is unavailable. Even fewer data have been published describing the pharmacodynamic effects of CoCl₂ administration in horses, particularly at high doses. The purpose of this pilot study was to describe the physiologic and biochemical effects of weekly intravenous doses of CoCl₂ to Standardbred horses. This report describes the hemodynamic effects of CoCl₂ in a dose escalation study.

Five Standardbred mares (12-13 years-old; 460-530 kg) were randomly assigned to receive one of 5 doses of CoCl₂ (4, 2, 1, 0.5, or 0.25 mg/kg) as an intravenous bolus (infused over 1 minute) once weekly for 5 weeks. Prior to each dose, animals were instrumented with pulmonary artery and right atrial catheters, a transverse facial artery catheter, two external jugular venous catheters, an indwelling urinary catheter, and electrocardiography leads. Physical examination parameters, blood pressure (systolic arterial pressure [SAP], diastolic arterial pressure [DAP], and mean arterial pressure [MAP]), cardiac output, and qualitative ECG assessment were evaluated every 5-10 minutes for 4 hours immediately after administration of the first and fifth weekly doses of CoCl₂.

All mares were subjectively anxious (nostril flaring, muscular tremor/fasciculation, pawing, straining) by 5 minutes following the CoCl₂ infusion; this persisted for ~60 minutes in mares receiving higher doses (4, 2, and 1 mg/kg). Mares receiving 4, 2, or 1 mg/kg doses developed tachycardia immediately after dosing (HR 60-126 bpm), but this was not observed in mares receiving 0.5 or 0.25 mg/kg (HR 36-52). Paroxysmal ventricular tachycardia was noted in the first 10 minutes post-administration in the mare receiving the 4 mg/kg dose. Elevations in SAP, DAP, and MAP were noted following drug administration at most doses; while profound hypertension was observed at 4 mg/kg (SAP/DAP, MAP [mmHg] = 291-300/163-213, 218-279), all mares became hypertensive in the 30-45 minutes following CoCl₂ administration. Mares receiving 4 and 2 mg/kg developed conspicuous oral mucous membrane congestion that persisted for 20 minutes post-dosing and subsequently resolved. At all doses, cardiovascular parameters returned to baseline by 1-2 hours post-administration.

Results of this preliminary study document significant, repeatable hemodynamic alterations associated with intravenous CoCl₂ administration to horses. Further, the degree of hypertension observed following infusion raises humane and human safety concerns if doses of >1 mg/kg are used.

Keywords: Cobalt, Horses, Cardiovascular, Hypertension, Pharmacodynamics
Performance enhancement

RESEARCH PATHOLOGY SUPPORT FOR EXPERIMENTAL ANIMAL MODELS PROVIDED BY THE COMPARATIVE PATHOLOGY & MOUSE PHENOTYPING SHARED RESOURCE. K. La Perle, S. Knoblaugh, J. Bolon, A. Flechtner, F. Jaynes, J. Rectenwald, A. Saulsbery, J. Sneddon. Department of Veterinary Biosciences.

The Comparative Pathology & Mouse Phenotyping Shared Resource (CPMPSR) at The Ohio State University supplies readily available, affordable, expert experimental pathology support to investigators utilizing animal models of human and veterinary disease. The CPMPSR comparative pathologists are familiar with the normal anatomy, physiology, and pathology of many animal species, including the potential impact of confounding factors such as age- and strain-related background lesions, pathogens, and husbandry practices on study outcomes. Primary research interests for the CPMPSR pathologists encompass cancer biology, developmental pathology, endocrine disease, immune-mediated conditions, neurobiology, and toxicologic pathology. However, translational research based on any animal model is supported. The CPMPSR offers a full array of pathology services, and can tailor its support to the needs of a client. Routine procedures include comprehensive macroscopic and microscopic examinations with an emphasis on phenotype characterization of newly produced lines of genetically engineered mice as well as pre-clinical efficacy and toxicity studies. Other common methods include clinical chemistry, hematology, radiography, whole slide digitization (Aperio) and quantification, frozen and paraffin slide preparation, transmission electronic micrograph grid and tissue microarray preparation, and many special histochemical and immunohistochemical staining techniques. The CPMPSR pathologists and staff are valuable collaborators for all facets of animal model development including study design, optimal sample collection, data analysis and interpretation, and communication. The CPMPSR was created to serve the experimental pathology needs of investigators at The Ohio State University, especially those in the seven health-related schools and the Comprehensive Cancer Center. However, the CPMPSR also functions as a referral service for experienced biomedical scientists at many other institutions (academic, government, and industrial).

Keywords: animal model, genetically engineered mice, histology, pathology, pre-clinical